Short- and long-term trade-offs in the diet selection of sheep

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Dedication

This work is dedicated to my parents,
Jean and Bill,
for their encouragement, love and support.

"Whatever you do, you need courage. Whatever course you decide upon, there is always someone to tell you are wrong. There are always difficulties arising, which tempt you to believe that your critics are right. To map out a course of action and follow it to the end requires some of the same courage, which a soldier needs. Peace has its victories, but it takes brave men to win them".

Ralph Waldo Emerson (1803-1882)

Declaration

I, hereby declare that this thesis is of my own composition, and that all assistance from other people has been duly acknowledged. The results presented herein have not previously been submitted for any other degree of qualification.

Sarah McDonald James

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Abstract

The purpose of this thesis was to investigate, in a series of 5 experiments, the basis of the diet selection of sheep, with the aim of shedding some light on the rules that ruminants might use when selecting their diet. This was done by investigating the effects of offering foods with rapidly degradable nitrogen and rapidly fermentable carbohydrates, within the rumen, on the food intake and diet selection of sheep.

Experiment 1 investigated whether sheep select between foods that differ in their effective rumen degradable protein (eRDP) content to achieve an estimated 'optimal' eRDP: fermentable metabolisable energy (fME) ratio. The foods offered were presented in a meal form to prevent chemical changes to the food ingredients that may occur during the pelleting process. However, as the food ingredients separated during the course of the experiment and the composition of the selected diet could not be sufficiently predicted, the results of the experiment were unclear.

Experiments 2 and 3 re-asserted the hypothesis that diets selected by sheep would be those that meet the eRDP requirements of the animals and at the same time avoid excess consumption of rumen degradable protein (RDP). The sheep were given choices between pelleted foods differing in their urea content, which was used as an eRDP source. However, sheep selected a diet in favor of the urea-supplemented food regardless of the basal food to which the urea was added. This resulted in an excess consumption of RDP. The results did not support the hypothesis that sheep would avoid excess RDP when given a choice and suggest that eRDP may not be a relevant dimension in diet selection in the conditions of these experiments. It was thought that urea may have had other properties, such as a high buffering potential, that the sheep were selecting for when selecting a diet.

Experiment 4 tested the hypothesis that the preference for an eRDP adequate food supplemented with urea would be reduced by the addition of a buffer (sodium bicarbonate (SB)) to both foods offered as a choice or offering *ad libitum* access to hay. In both cases urea may no longer be needed as a buffer and the preference for

the urea-supplemented food would then be expected to be reduced or even disappear. Insufficient amounts of hay were consumed to reduce the preference for the urea-supplemented food. Supplementing both foods with SB significantly reduced the preference for the urea-supplemented food. It was proposed that the effect of SB addition on the diet selection of sheep was carried though its buffering properties. Therefore it could be hypothesized that the sheep in this experiment, and those in the previous two, were prepared to over consume RDP, when supplied by urea, due to the buffering potential of the urea supplemented food. When this excess consumption was made unnecessary by adding SB, sheep avoided an excess intake of RDP. The idea that sheep might give greater weighting to the benefits of maintaining their rumen within a desired physiological range rather than avoid an excess consumption of RDP, led into the second line of research of this thesis.

The final experiment investigated whether a large rather than small change within the rumen environment, due to the consumption of a 'disruptive' food (i.e. food that would lower rumen pH), would alter subsequent diet selection. Sheep were offered high or low energy density (ED) foods with or without SB for a period of 2 hours. All sheep were then offered a choice between the high and low ED foods unsupplemented for a further 6 hours. SB supplementation did not alter subsequent diet selection. The magnitude of change in the pH of the rumen may not have been sufficient to alter subsequent diet selection. However, sheep initially offered the high ED food subsequently selected more of the low ED food during the 6 hours when a choice was offered compared to sheep initially offered the low ED food. The results from this experiment agree with the hypothesis that changes within the rumen of large, rather than small, magnitude due to the consumption of a disruptive food alters subsequent diet selection.

Taken together, the results from these experiments are in agreement with the hypothesis that one of the objectives of the diet selection of ruminants is to maintain the rumen conditions within a certain physiological range. Therefore, in the absence of long forage, ruminants would be expected to select foods with the greater buffering potential firstly to prevent further disruption to the rumen environment and

secondly to return the conditions in the rumen to within the accepted range as soon as possible. This need appeared to override other diet selection objectives such as avoiding an excess intake of RDP. However, the basis of protein selection by ruminants still remains unclear. Further research is required to strengthen the argument that ruminants select a diet to avoid both deficient and abundant concentrations of eRDP.

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Chapter 1

General Introduction

The first and simplest emotion, which we discover in the human mind, is curiosity.

Edmund Burke (1729-1797)

1.1 Introduction

In natural environments, there is a variety of foods available that herbivorous animals are able, and prepared, to eat (Westoby, 1974; Armstrong *et al.*, 1997 a, b). However, of the many foods available, when consumed alone, few will provide all the nutrients essential for the growth, development and reproductive functions of the animal (McDonald *et al.*, 1995). In addition, many foods contain anti-nutritional factors that are harmful if consumed in large quantities (Rosenthal and Janzen, 1979; Blowey, 1993). The animal can thus be seen as facing two problems: (i) what foods to eat, and (ii) how much of each food to eat (Kyriazakis, 1997). Animals, including ruminants, appear to have developed dietary strategies that allow them to meet their nutritional needs, yet reduce the likelihood of over-ingesting toxins. For example, grazing ruminants have been shown to select a diet that contains higher energy (Newman *et al.*, 1992; Parsons *et al.*, 1994; Penning *et al.*, 1997), and lower toxin, content (Kronberg and Maleckek, 1997; Duncan and Gordon, 1999) than that which would be ingested if diet selection were random. It can therefore be assumed, and has been suggested by many authors (e.g. Kenney and Black, 1984; Van Wieren, 1996; Kyriazakis *et al.*, 1999), that an animal will use a feeding strategy that will maximise the benefits (e.g. nutrients and energy) and minimise the costs (e.g. toxins and energy expenditure) of a diet. Food choice affects both benefits and costs. One choice may have both higher benefits and higher costs than another choice and the animal will then have to make a trade-off between the increased benefits and costs in order to come to a decision in relation to its diet selection. An animal can be assumed to make the choice that maximises the difference between benefits and costs (Kyriazakis *et al.*, 1999).

The study of the diet selection made by animals in their natural environment is arduous, due to the difficulty in adequately describing the feeding, thermal and social environments (Westoby, 1974; Emmans, 1991). Diet selection can however be studied in controlled environments, where the composition of foods offered and the environments can adequately be described. The study of diet selection under such conditions has been used with a considerable degree of success on a number of

species such as laboratory rodents (Leshner *et al.*, 1972;White *et al.*, 2000), poultry (Emmans, 1977, 1980; Shariatmadari and Forbes, 1993; Macleod and Dabutha, 1997), pigs (Bradford and Gous, 1991; Kyriazakis and Emmans, 1992), and sheep (Cropper, 1987; Kyriazakis and Oldham, 1993). Simple experiments, such as these, are necessary first steps to determine what dimensions are relevant to the animals when they select a diet. The common assumption made in such experiments is that, even under artificial conditions, the diets selected by the animal will reflect the general adaptive nature of feeding behaviour (Siegel, 1993). The results from these experiments can help to develop frameworks that allow diet selection to be explained and predicted in more complex situations such as those found in grazing and natural systems.

The aim of this chapter is to review the existing literature that provides a background to the study of food intake and diet selection for protein in both non-ruminants and ruminants. This chapter will subsequently focus on the diet selection of ruminant animals, as this is the subject of this thesis. In doing so, this chapter will also discuss the complexity of the ruminant's digestive system and how the rumen metabolism, and the attributes of food, affect food intake and diet selection by ruminants.

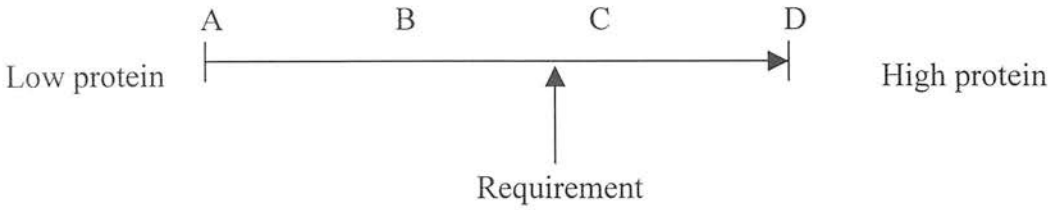
1.2 Theory of diet selection

The principles of diet selection have been outlined by Emmans (1991). Where the compositions of the foods offered as a choice are such that the animal can choose a diet to meet its nutrient requirements, the theory of diet selection states that the animal will do so. Where the composition of the foods offered as a choice are such that no mixture of them will allow an adequate diet to be selected, the theory of diet selection asserts that the animal will select a diet that minimises either deficiency or excess. The simplest case to consider is when an animal is given free and continuous access to two foods, which differ in only one dimension, as a choice. The proportions of each of the two foods offered as a choice, necessary, to meet the animal's requirements for the nutrient, for example protein, can be represented on a straight

line (Figure 1.1).

Foods A and B, in figure 1.1, are both deficient in protein in comparison to the requirement, food A more so than food B. Foods C and D both contain excess protein in comparison to the requirement, food D more so than food C.

Figure 1.1 *Diagrammatic representation of mixtures of foods required to satisfy an animal's demands for protein. A, B, C and D are foods on positions which represent their concentration of protein in relation to energy.*



Using the principles of diet selection described above, an animal offered a choice of A or B with either C or D would be expected to select a diet which is a mixture of both the deficient and abundant protein foods and therefore select a diet to meet its protein requirement. An animal offered A and B, as a choice, would be predicted to predominantly select B and hence minimise the protein deficiency. An animal offered C and D, as a choice, would be predicted to predominantly select C and hence minimise an excess intake of protein.

The theory of what animals should select to eat can be used only if the requirements of the animal(s) in question are known. To define the responses to protein under specific environmental conditions, it is preferable to conduct experiments with single foods of a range of protein concentrations, but this is not often done. Alternatively, estimates of protein requirements can be obtained from textbooks and tables (e.g. Agricultural and Food Research Council (AFRC), 1993; McDonald *et al.*, 1995). The models used to estimate the protein requirements of animals displayed in tables are

generally empirical, i.e. they have been derived from the data of a large number of experiments with different environmental conditions, different animal breeds within a species and animals of different physiological state, rather than from theory. Protein requirements can also be calculated directly from such models. Values such as live weight and weight gain, relevant to the animals under investigation, can be inserted into the models that estimate protein requirements to increase the accuracy of the prediction. The following section will briefly describe the current protein systems used to predict the protein requirements of animals and the protein supply from foods.

1.3 Protein systems that estimate protein requirements of animals and protein supply from foods

The nitrogen content of foods have been traditionally expressed as crude protein (N x 6.25). This approach is useful as a simple means of expressing the overall level of nitrogen in the food. However, the crude protein (CP) content gives no indication of how efficiently the protein is utilised. The digestible crude protein (DCP) content of foods can be determined via digestibility trials where it was assumed that the difference between the protein in the diet and faeces represented the quantity of protein absorbed. It has been recognised that there is a significant effect of rumen fermentation on the nutrient supply for ruminants and that micro-organism within the rumen can utilise non-protein nitrogen (NPN) to provide the ruminant host with protein. In addition, non-ruminants not only have a general requirement for protein, but also a specific dietary requirement for essential amino acids. With these in mind it becomes obvious that CP and DCP would not give an adequate description of the amount and nature of the protein that provides the organs and tissues with amino acids after absorption from the digestive tract.

1.3.1 *Protein systems for non-ruminants*

The protein requirements of non-ruminants have been estimated by a factorial

method in which the daily net requirement for protein is estimated as the sum of the requirements for maintenance, growth, and production such as pregnancy and lactation (Agricultural Research Council (ARC), 1981). Each of these needs is considered as a need for a certain quantity of each amino acid. However, the body does not use the amino acids supplied to it as individual entities, but rather for purposes of synthesising proteins from mixtures of essential and non-essential amino acids. The balance of amino acids supplied is therefore important. Amino acids supplied in excess cannot be used for protein synthesis and are deaminated (Lewis, 1991).

The ideal protein of a diet offered to non-ruminants can be described as the balance of amino acids that most closely approaches that necessary for maintenance, growth and production (Fuller and Chamberlain, 1985). The ideal protein has been derived from individual estimates of amino acid requirements (Lewis, 1991), and the results of experiments designed specifically for that purpose (Wang and Fuller, 1989). The suggested amino acid composition of ideal protein for growing pigs, and pregnant and lactating sows are given in Table 1.1 (ARC, 1981).

Having provided the criterion for ideal protein, the amino acid spectrum of the protein in a mixed diet can be compared with it and the value of the protein in the diet (V) derived. The effective value of the protein in the diet is that proportion of the dietary protein judged utilisable on the strength of the most limiting amino acid. For example, if a mixed diet offered contained 45g lysine/kg dietary protein, as in the fictional mixed diet displayed in Table 1.1, and lysine was the most limiting amino acid, the maximum efficiency of use of that dietary protein (V) would be 0.64 (amount in diet/amount required: 45 ÷ 70). Thus 64% of the ileally digested and subsequently utilisable protein would be available (although not necessarily all used) for maintenance and for protein tissue synthesis, while 36% deaminated and excreted in the urine.

If the animal had a calculated requirement for 0.24kg of utilisable ideal protein per day and was offered the fictional mixed diet displayed in Table 1.1, using the above

utilisable protein value (V) of 0.64, the animal would need to consume at least 0.375kg each day (0.240 ÷ 0.64). The addition of artificial lysine to the diet would raise the lysine concentration of the protein. This would result in the use of the next limiting amino acid (in the case of the fictional diet this would be Threonine) needing to be used to predict the effective value of the protein and hence required food intake. The addition of artificial lysine to a diet where lysine is most limiting would therefore raise the value of utilisable protein and subsequently result in the animal not needing to consume as much CP daily.

Table 1.1 *Derivation of the protein value (V) of a mixed diet (Whittemore, 1998).*

	Ileal digested and utilisable amino acid (g/kg diet protein) (A)	Amino acids (g/kg ideal protein) (B)	V (A/B)
Lysine	45	70	0.64
Methionine + cystine	30	35	0.86
Threonine	30	42	0.71
Tryptophan	10	10	1.00
Iso leucine	35	38	0.92
Leucine	70	70	1.00
Histidine	20	23	0.87
Phenylalanine + tyrosine	65	67	0.97
Valine	40	49	0.82

1.3.2 *Protein systems for ruminants*

For ruminants, the total digestible true protein (amino acid) requirement, expressed as metabolisable protein (MP), is calculated using a factorial approach similar to that of the non-ruminants. That is, the total MP requirement is calculated by the summation of the MP required for each body process such as maintenance, growth and production. However, unlike the estimation of requirements for non-ruminants, only the total metabolisable protein is estimated, and as yet, not the individual requirements for essential amino acids. However, though not in use, equations

predicting requirements of individual amino acids and the supply of amino acids from the food have been formulated (O'Connor *et al.*, 1993; Chalupa and Sniffen, 1994).

The MP supply comes from two sources (Figure 1.2): the digestible microbial true protein (DMTP) and the digestible undegradable food protein (DUP). Digestible microbial true protein is the protein yielded from the digestion of microbial cells, leaving the rumen, within the small intestine. About 25% of the microbial crude protein (MCP; see below) is present as nucleic acids and cannot be used by the ruminant host. It has been estimated that about 85% of the MCP is digestible in the intestines. Therefore:

$$\text{DMTP (g/d)} = 0.75 \times 0.85 \times \text{MCP} = 0.6375 \text{ MCP (g/d)}$$

(AFRC, 1993)

Digestible undegradable protein is the fraction of food protein that has not been degraded within the rumen, but is available for digestion within the lower intestines of the animal. Therefore MP yield is defined as:

$$\text{MP (g/d)} = 0.6375\text{MCP} + \text{DUP}$$

(AFRC, 1993)

1.3.2.1 *Microbial protein synthesis in the rumen*

Food proteins are hydrolysed to peptides, amino acids and ammonia by rumen micro-organisms. The ammonia produced (together with some small peptides and free amino acids) is utilised by the rumen micro-organisms to synthesise microbial protein (Figure 1.2). Micro-organisms can also utilise NPN supplied either from the diet or recycled from the blood, in the form of urea, via the saliva or diffusion across the rumen wall, to synthesis microbial protein (Leng and Nolan, 1984; Brun-Bellut, 1996). The total nitrogen supply, multiplied by 6.25, actually utilised by the rumen micro-organisms for growth and synthetic purposes is know as the effective rumen degradable protein (eRDP). AFRC (1993) suggested that eRDP is used with the

efficiency of 1.0 for microbial protein synthesis. Effective rumen degradable protein comprises of both quickly (QDP) and slowly (SDP) degradable protein. The QDP fraction of the total crude protein, comprising considerable amounts of NPN and small protein molecules, is released rapidly when the foods enter the rumen, resulting in an efficiency of capture by the rumen microbes of less than one. An efficiency of 0.8 has been assigned to the QDP fraction of foods (ARC, 1980). The SDP fraction of the total crude protein is released more slowly after food enters the rumen. Therefore, ARC (1980) assumed a net efficiency of capture for protein N of 1.0. Therefore:

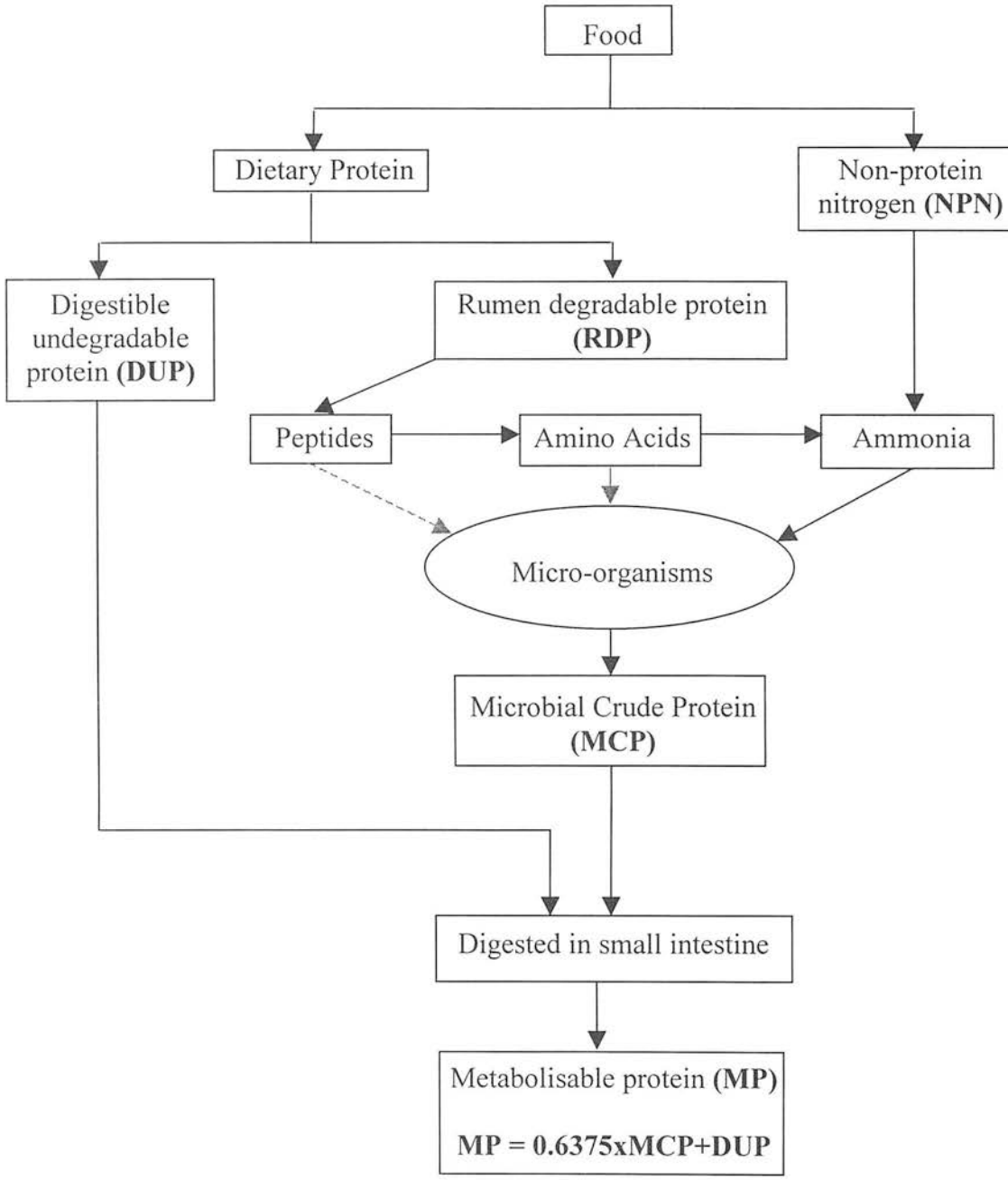
$$\text{ERDP (g/kgDM)} = 0.8[\text{QDP}] + [\text{SDP}]$$

(AFRC, 1993)

Micro-organisms also require a supply of fermentable metabolisable energy (fME) to synthesise protein. The fME content of a food is defined by AFRC (1992) as the metabolisable energy (ME) content of the food, as MJ/kg DM, less the ME present as total oils and fats and the ME contribution of fermentation acids present in partially fermented forages such as silage. The amount of eRDP captured and utilised by the microbes, and therefore MCP yield, is set by the availability of fME (Russell *et al.*, 1983), if eRDP is abundant. Therefore, the MCP yield is expressed as g MCP per MJ of fME in the diet.

The efficiency of bacterial growth per MJ fME available is influenced by outflow rate (Meng *et al.*, 2000). Outflow rate in turn is influenced by level of feeding. It has been estimated that for maximal protein synthesis, rumen micro-organisms of animals fed at maintenance require 1 MJ of fME to capture and utilise 9g eRDP and hence synthesise 9g MCP. Growing sheep and cattle, which are considered to have a feeding level between one and two times maintenance, are predicted to synthesise 10g MCP/ MJ fME and therefore require 10g eRDP/ MJ fME (ARFC, 1993). As the level of feeding increases, so does MCP synthesis per MJ fME.

Figure 1.2 *Digestion and metabolism of nitrogenous compounds in the rumen.*



If RDP supply is less than the RDP requirement set by the fME content of the food, then the diet is RDP limited and MCP yield is equal to RDP supply. If RDP supply exceeds RDP requirement, then fME is the first limiting MCP yield and MCP is equal to the supply of fME multiplied by the yield of MCP synthesised per MJ fME.

1.3.3 *Future systems for estimating protein supply to the ruminant*

The concepts included in the AFRC (1993) protein system are scientifically defensible and recognise the energy and protein interactions, albeit only at the rumen level. Other protein systems such as the PDI (Protein truly digested in the small intestine) system (INRA, 1989) used in France also recognises that the protein supply for ruminants comes from the two sources, DUP and MCP and also takes into account the energy and protein interactions within the rumen. The method of calculating protein yield within the small intestine and a number of the standard coefficients used, such as the efficiency of eRDP use, differ between the two systems. However, although these system, based on the same ideas, are generally regarded as an improvement to the CP and DCP system traditionally used, areas needing further refinement remain (Meehan *et al.*, 1996). Such refinement includes the accurate prediction of the MCP supply to the host.

At present there are major uncertainties regarding the yield of MCP (Webster, 1996). Therefore, new mechanistic models are required where discrepancies in the current systems are approached. Mechanistic models are constructed by looking at the structure of the system in question, dividing the system into its key components and analysing the behaviour of the whole system in terms of its individual components and the interactions between the individual components (France and Thornley, 1984). The prediction of MCP supply is based on knowledge of individual components of rumen function which include degradation of substances in the rumen, the efficiency of growth of rumen micro-organisms and the metabolism of individual microbial species (reviewed by Dijkstra and Bannink, 1999). There is evidence available on each of these components such as databases containing information on *in vivo* protein and carbohydrate degradation of various foods (Tamminga *et al.*, 1990) and knowledge on the micro-organisms (Hobson, 1988; Hobson and Stewart, 1997). One of the tasks of the modeller is to extract the data required for the specific objective of the model. Not surprisingly, a range of models exist to predict MCP supply.

In order to accommodate some of the limitations of the existing protein systems, the Cornell net carbohydrate and protein system (CNCPS) was developed (Fox *et al.*, 1992; Russell *et al.*, 1992; Sniffen *et al.*, 1992). This system has a number of mechanistic components, like sub-models that estimate the quantity of end products, such as MCP, and therefore is attractive for further development of current protein evaluation systems for ruminants. However, although the CNCPS system recognises that the fibre content of the food consumed affects rumen pH, additional work is required to relate rumen pH with fibre degradation rate (Russell *et al.*, 1992). As the products of fibre digestion are a major source of energy for microbial protein synthesis, proper representation of fibre degradation is vital for accurate prediction of MCP supply. However, to date, the models of the effects of pH upon fibre degradation based on both *in vitro* and *in vivo* data, differ extensively (e.g. Dijkstra *et al.*, 1992; Lescoat and Sauvant, 1995; Pitt *et al.*, 1996). In vivo, the pH below which fibre degradation is reduced is approximately 6.3 (reviewed by Erdman, 1988). According to Pitt *et al.* (1996) the fractional degradation rate of fibre at pH 5.5 is about 38% of its maximum rate. However, Dijkstra *et al.* (1992) and Lescoat and Sauvant (1995) estimate that the fractional degradation rate of fibre at pH 5.5 is about 16 and 50% of its maximum rate respectively.

The prediction of individual components, such as the effect on pH upon fibre degradation, using mechanistic models currently may be highly variable. However, mechanistic models are very useful in evaluating the adequacy of current knowledge and identifying areas where research efforts should be focused (Forbes and France, 1993).

1.4 Regulation of protein intake

The results from experiments addressing the ability of non-ruminants to regulate their protein intake are generally consistent with the hypothesis that non-ruminants attempt to regulate their protein intake to meet their protein requirements and avoid an excess of surplus protein. However, there is some variability in the results of

experiments that may reflect the differences in the methodology used. The evidence from ruminant animals however, has not always been consistent with the above hypothesis. The inconsistency of results from experiments looking at the ability of ruminants to regulate their protein intake may be due, not only to differences in the methodology used but, to the complexity of the ruminant digestive system in dealing with protein and hence the estimation of MP. The following section will review the literature on the regulation of protein intake by non-ruminants and ruminants. It will try to determine if the variability in the results from experiments are due to variations in the methodology used, and in the case of ruminants, in the difficulties in interpreting whether protein regulation should be interpreted in terms of MP or the RDP axis.

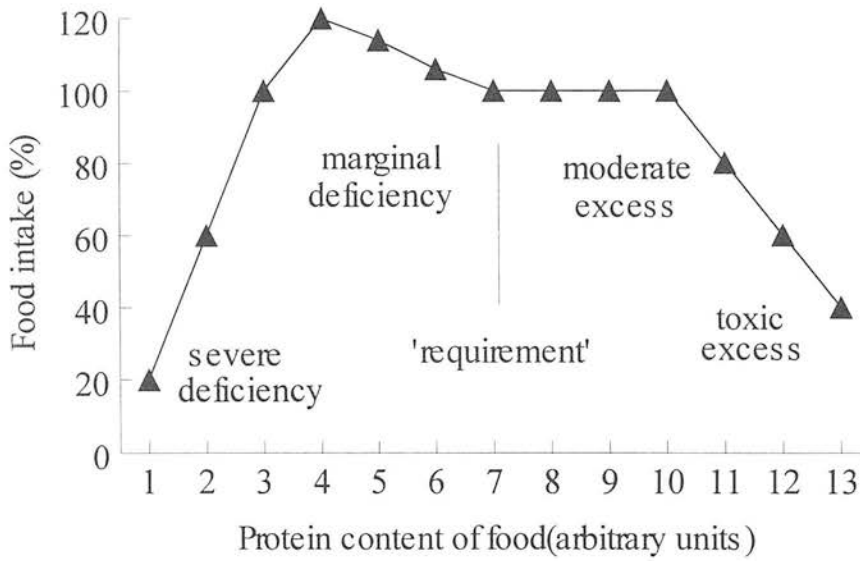
1.4.1 *The effect of food protein content on intake by non-ruminants*

An animal, given free and continuous access to a single homogenous food, over a period of time, can vary its protein intake only by eating more or less of the food offered (Kyriazakis, 1997). When the protein content of the food decreases to below the optimum for growth, there is often a modest increase in food intake (Figure 1.3) as the animal tries to maintain its intake of protein (Musten *et al.*, 1974; Kyriazakis *et al.*, 1990, 1991). The animal would be expected to cope with the relative excess of other nutrients and energy by excreting them, depositing them as fat or by increasing heat production until a capacity for doing so is reached. Kyriazakis *et al.* (1991) offered pigs foods differing in protein contents whilst the energy content remained relatively constant. As the protein content decreased below the optimum for growth, food intake increased. The extra energy consumed was deposited as fat and dissipated as heat. However, compensation in protein intake was not completely achieved, as the weight gain of these pigs was lower than that of pigs offered foods with higher protein contents. This was assumed to reflect the fact that the maximum ability of the pigs to dissipate heat in that environment had been reached.

As the protein content of the food declines further, the animal would need to consume more of the food to meet its protein requirements. The bulk of the food or

the ability of the animal to dissipate the heat from the excess energy consumed may prevent the animal from doing this. Therefore food intake can be expected to decrease since the animal would be unable cope with the severe imbalance in the nutrient supply (Likuski *et al.*, 1961; Shariatmadari and Forbes, 1993).

Figure 1.3 *Generalised diagram of the effects on voluntary food intake of excesses and deficiencies of protein in food (Forbes, 1995).*



Non-ruminants do appear to be able to cope with the mild over-provision of protein and within a certain range of food protein contents, food intake is not affected (Kyriazakis *et al.*, 1990). The excess amino acids consumed can be deaminated within the liver although this does produce urea and heat. As the protein content of the food increases further there may be substantial effects on the energy and nitrogen metabolism of the animal (Lobley *et al.*, 1995). Food intake can be expected to decrease when the protein content of the food offered is very high (Latshaw, 1993). A severe excess of protein can lead to increased heat production from the deamination of the excess amino acids. The increased heat production may depress food intake if heat dissipation is limiting (Ferguson and Gous, 1997). In addition the

products of deamination may become toxic to the animal resulting in a decrease in food intake.

1.4.2 *Selection between foods of different protein contents by non-ruminants*

An animal, given either free and continuous access to a single heterogeneous food, or a choice of foods, can regulate its protein intake by choosing what part of the food to consume, or in the case where a choice is offered, which food, and how much of it, to consume (Kyriazakis, 1997).

There is now substantial evidence to support the idea that non-ruminant animals are capable of choosing a diet, from two or more foods, to meet their protein requirements. The majority of experiments offered animals two foods, one with an abundant amount of protein, the other with a deficient concentration of protein relative to requirements respectively. The animals chose a non-limiting combination of the two foods and avoided an excess intake of protein, as demonstrated by the performance of animals offered single foods (Leshner *et al.*, 1972; Musten *et al.*, 1974; Kyriazakis *et al.*, 1991; Shariatmadari and Forbes, 1993).

Foods with mildly deficient protein contents can result in an increase in food intake, where a severely deficient food would result in a decrease in food intake (Figure 1.3). Therefore it would be advantageous for the animal to predominantly select the less limiting food, and hence minimise the deficiency, when offered a choice between two foods both deficient in their protein content. This has been shown to be the case in poultry (Shariatmadari and Forbes, 1993) and rats (Musten *et al.*, 1974). However, in some cases the animals do not opt to consume predominantly the food that was least limiting, but included a large proportion of the more limiting food in their diet (Holcombe *et al.*, 1976; Kyriazakis *et al.*, 1990). It was suggested that this was due to the animals continuously sampling both foods available. This continuous monitoring of the composition of both protein deficient foods offered would allow the animal to rapidly change its diet selection should the protein content of one of the foods offered increase.

As described above, non-ruminants can cope with mild excess in the protein content of the food, but food intake decreases when the protein content of the food is very high. Therefore animals would be predicted to show a preference for the food containing the lower protein content so that an excess protein intake is minimised. The diet selection of non-ruminants appears to follow this prediction (Kyriazakis *et al.*, 1991; Shariatmadari and Forbes, 1993; Forbes and Shariatmadari, 1996).

However, not all investigators have suggested that animals can choose a diet to meet their protein requirements. Galef (1991) quoted 'Failure to compose an adequate diet from a cafeteria of foods are informative in themselves and that such failure provide *prima facie* evidence of an inability of animals to select a diet to meet their requirement'. When results from diet selection experiments are initially examined, it may appear that the animals are unable to select a diet to meet their requirements. However, there are a number of conditions required for a diet selection experiment. If these conditions are not met the animal would be unable of select an appropriate diet.

There are a number of conditions needed for diet selection to be properly exercised such as: i) sufficient time to allow the animals to learn to associate sensory, or other properties, with the nutrient contents of the foods offered, ii) adequate foods being offered as a choice and iii) toxin contents in the foods offered minimised (Forbes, 1995; Forbes and Kyriazakis, 1995). When experiments do not meet the conditions required for diet selection to be properly exercised, the animals might not be able to select a diet that meets their protein requirements.

Sufficient time is required for the animal to learn to associate sensory, or other, properties with the nutrient contents of the foods offered (Krebs and McCleery, 1984). Altering the position of the foods too frequently can 'trick' the animal into selecting an inappropriate diet, as animals can use cues, such as position to associate which foods results in what postingestive consequences (Forbes and Kyriazakis, 1995). Frequent changing of the food position can result in the animal failing to

choose a diet to meet its requirement (e.g. Vohra and Heil, 1969). Therefore, results from experiments that have altered food position too rapidly may have concluded an inability of the animal to select an appropriate diet whereas the animal simply may not have been offered the opportunity to do so (Wood-Gush and Kare, 1966).

As described in the theory of diet selection section, for the animal to select a diet to meet its requirements, the nutrient content of the foods offered as a choice need to allow the animal to do so. That is, when two foods are offered as a choice, one food needs to be above and the other food below the requirement of the animal for an appropriate diet to be selected. Should the foods offered not meet this criteria, no combination will allow the animal to select a diet to meet its requirement (Cowan and Michie, 1977; Nam *et al.*, 1995). It is therefore important to use control animals in diet selection experiments so that the performance of the choice group animals can be compared with animals offered only one of the foods offered as a choice (Musten *et al.*, 1974; Kyriazakis and Oldham, 1993). Although this seems to be an obvious condition that needs to be met in order to observe the diet selection for protein, it is surprising how many times this has been violated.

Finally, animals may not be able to select an appropriate diet to meet their requirements due to the presence of toxins, such as glucosinolates present in rapeseed meal, in one of the foods offered as a choice. Kyriazakis and Emmans (1992) showed that growing pigs, given choices between foods of different protein contents, that also varied in their toxin content, avoided foods containing rapeseed meal, hence the glucosinolates. This resulted in over- and under-consumption of protein when the rapeseed was in the low and high protein foods respectively. Similar results have been demonstrated by Gill *et al.* (1995).

The evidence from non-ruminant animals suggest that animals are able to select a diet which reflects their protein requirement whilst at the same time avoids an excess consumption of protein when offered an 'appropriate' choice of foods and sufficient time is allowed for the animals to learn about the foods offered as a choice (Gibson and Booth, 1986; Baker *et al.*, 1987).

1.4.3 *The effect of food protein content on intake by ruminants*

Ruminants appear able to cope with mild under as well as mild over provision of protein, and within certain range of food protein contents, food intake in not affected (Ørskov *et al.*, 1971; Kyriazakis and Oldham, 1993). It has been perceived that, due to the utilisation of NPN from recycled urea, by micro-organisms, to synthesise microbial protein, ruminants are, in the short-term, less restricted by the quantity of protein required to be consumed (Elliott and Topps, 1963).

However, unlike the monogastrics, ruminants frequently do not attempt to compensate when offered a food of low protein content by increasing their food intake (Ørskov *et al.*, 1971; McGregor and McLaughlin, 1980; Kyriazakis and Oldham, 1993; Ternouth *et al.*, 1993). The absence of compensatory intake in ruminants offered a low protein food has often been suggested to be a result of the rumen capacity limiting food intake. Low protein foods are usually created by diluting a high protein food with increasing amounts of fibrous material (Raven *et al.*, 1969). Hence as the fibrous material content increases, physical limitation restricts further intake (Forbes, 1995). In addition to this, nitrogen is required by the micro-organisms for maintenance and growth (Al-Rabbat *et al.*, 1972; Ørskov, 1992). When the supply of nitrogen is deficient, and the supply of recycled urea depletes, the microbial activity reduces and microbial population depletes (Kanjanapruthipong and Leng, 1998; Carro and Miller, 1999). As a result, the capacity to ferment the foods ingested decreases and fibrous foods then remain undigested within the rumen again resulting in a physical limitation on food intake (Ørskov *et al.*, 1971; Ternouth *et al.*, 1993).

Many experiments have also observed such a decrease in food intake of low protein diets when the diet has been a high energy density, highly digestible concentrate (e.g. Kyriazakis and Oldham, 1993). Concentrate foods are readily fermentable and most yield more volatile fatty acids (VFAs), the end product of carbohydrate fermentation, per unit mass than forages (Baik *et al.*, 1997). The rapid ingestion of readily fermentable carbohydrates can result in a decrease in the rumen pH, which is

frequently associated with a reduction in food intake (Counette *et al.*, 1979; Forbes and Barrio, 1992) possibly as a result of decreased organic matter digestion (Newbold, 1987). Increasing food intake to compensate for the low protein content of the diet offered would result in an increase intake of readily fermentable carbohydrates, and hence increases in VFA concentration within the rumen. Due to the negative effects of a high VFA concentration and low pH within the rumen on food intake, as described in section 1.5, this would be counter-productive to the animals attempting to increase protein intake.

Further decreases in the protein content of the diet offered to ruminants results in a decrease in food intake as observed in non-ruminants (Ørskov *et al.*, 1971; Egan and Doyle, 1985; Kyriazakis and Oldham, 1993; Tolkamp *et al.*, 1998a). The bulk of the food and the ability of the animal to cope with the imbalance between nitrogen and energy supply may cause the animal to reduce its food intake.

Various authors have shown that supplementing a food deficient in eRDP with urea, a source of RDP, stimulates microbial activity, increases the rates of intake (Egan and Doyle, 1985; Manyuchi *et al.*, 1994) and live weight gain (Ørskov *et al.*, 1972; Kyriazakis and Oldham, 1993). However, when the concentration of urea added is high (Nomani, 1973), or when animals have restricted access to the urea supplemented foods (Bloomfield *et al.*, 1961; Campbell *et al.*, 1963), food intake is reduced. Wilson *et al.* (1975) showed that urea poured into the rumen as a solution, two times each day, resulted in a significant decrease in food intake. The fall in intake was less when the same quantity of urea was continuously infused over 24 hours or supplemented to the food offered *ad libitum*. It appears that ruminants try to avoid an excess consumption of RDP, especially rapidly degradable RDP such as urea, which could be an attempt to reduce the rise of ammonia concentration in the rumen (Villalba and Provenza, 1997; Tolkamp *et al.*, 1998b).

The beneficial effects of urea, supplemented to an eRDP deficient food, on food intake and weight gain are however limited (Ørskov *et al.*, 1972; Shain *et al.*, 1998). Although several studies (Nolan and Leng, 1972; Russell *et al.* 1983) have shown

that the mixed bacterial population use ammonia as their main source of nitrogen, pre-formed amino acids and peptides are also required (Carro and Miller, 1999). The supplementation of dietary protein has been shown to enhance food intake to a greater extent than urea when added to a protein deficient food (Koster *et al.*, 1997) by stimulating microbial activity to a greater extent (Cotta and Russell, 1982; Griswold *et al.*, 1996) and subsequently increasing organic matter digestion (McAllan, 1991).

A lot of work has looked at ways of overcoming microbial degradation of dietary protein by using DUP, or by treating dietary protein with formaldehyde to render the protein undegradable. However, increasing DUP supply has often resulted in a lack of response in food intake and performance of the animal (Hunter and Siebert, 1987; Merchen *et al.*, 1987; Sloan *et al.*, 1988; Clark *et al.*, 1992). Digestible undegradable protein and by-pass protein may have had poor essential amino acid profiles, or result in an amino acid imbalance supplied to the host (Santos *et al.*, 1998) and therefore may not have had any beneficial effect (Robinson *et al.*, 2000).

Digestible undegradable protein may be of importance when eRDP is limiting (Mishra and Rai, 1996). The amino acids absorbed from the digested DUP can be de-aminated resulting in a production of urea. This urea can then be recycled to the rumen and provide nitrogen for microbial protein synthesis and hence increase microbial activity and subsequently result in an increase in food intake. Additional to this, DUP may be of importance when the microbial protein yield does not meet the requirements of the host such as in the case of the high-yielding dairy cow (Bruckental *et al.*, 1989). In this circumstance, the additional supply of amino acids to the small intestine, provided by additional DUP to an eRDP sufficient food, may result in an increase in production.

There is a lack of experimental evidence that demonstrates the ruminants will decrease their food intake of diets that supply very high concentrations of digestible protein to the small intestines, i.e. foods containing high concentrations of DUP. However, it has been demonstrated that, as the DUP content of the food offered

increases, the blood urea concentration also increases (Bruckental *et al.*, 1989; Cody *et al.*, 1990). The excess amino acids absorbed from the small intestine may therefore be deaminated within the liver producing urea and heat, and as the DUP content of the food increases further, may have substantial effects on the energy and nitrogen metabolism of the animal (Lobley *et al.*, 1995). It could therefore be postulated that the food intake of ruminants, like non-ruminants, would decrease when the MP yield from both DUP and MCP is very high. This decreased food intake may be due, not only to the ruminant attempting to avoid high ruminal ammonia levels, but also to an increased heat production or the products of deamination becoming toxic to the animal.

Unlike monogastrics, ruminants do not increase their food intake as the protein content of the food offered decreases. However, they do appear to try to avoid and excess consumption of RDP, especially when in the form of NPN. The following section outlines some of the research on the ability of ruminants to select a diet to meet their protein requirements from a choice of foods. This section will also go through a series of experiments that looked at what dimensions the ruminants appear to select for with regard to protein.

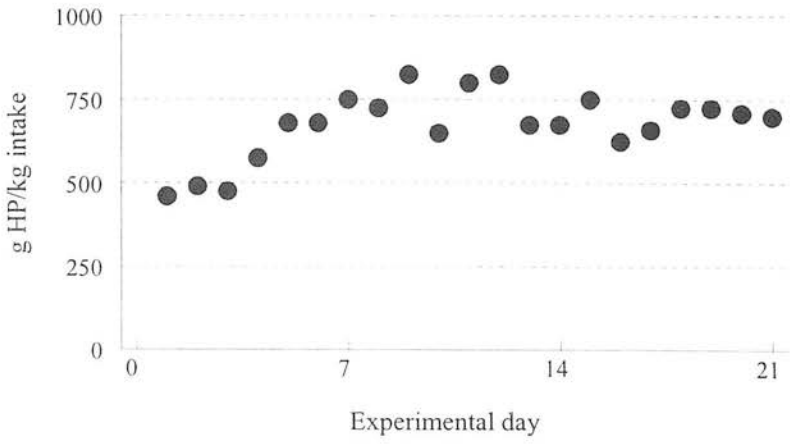
1.4.4 *Selection between foods of different protein contents by ruminants*

There is increasing evidence to suggest that ruminants may be capable of selecting between two foods, differing in their protein content, to meet their protein requirements. Cropper (1987), Hou (1991), Kyriazakis and Oldham, (1993) and Gorgulu *et al.*, (1996) found that, in a controlled environment, growing sheep selected a diet to meet their MP requirements from two foods that differed mainly in their MP yields. The test of adequacy was the performance of similar sheep on a series of single foods (Emmans, 1991). These results were thus consistent with those on non-ruminant animals despite the complexity of the ruminant digestive system. However, in the above experiments there were differences between the foods used in both their calculated MP yields and in their RDP contents, and it was not clear whether the diet selection observed in these experiments should be interpreted in

terms of MP or the RDP axis. The following experiments demonstrate the difficulty in interpreting the dimension(s) relevant to ruminants with respect to protein when selecting a diet.

The majority of diet selection experiments on ruminants looked at the ability of sheep to select a diet to meet their protein requirements. An experiment was initiated to find out if cows were able to detect differences in the protein content, in otherwise similar foods, and select a diet to meet their MP requirements (Tolkamp and Kyriazakis, 1997). Two foods were formulated both of which were a mixture of grass silage and concentrates with the same silage content on a DM basis. The concentrates of the two foods differed so as to result in a low MP food (LP, 82g MP/kg DM) and a high MP food (HP, 109g MP/kg DM) but with similar fME and ME values. These foods were offered to groups of eight cows, one group of which received access to both foods as a choice from day one of the experimental period. For the first three days, the mean diet selected did not differ from random. Subsequently, the groups mean increased (Figure 1.4); all cows showed a preference for HP. In addition, cows offered the choice of foods attained similar food intakes and milk yields to a control group offered HP alone. Cows offered LP alone did not.

Figure 1.4 *Diet selection of cows getting access to two novel foods. One food (HP) had 109g MP/kg DM and one, LP, had 82g MP/kg DM. Diet selection was expressed as g HP consumed/kg total food intake (Tolkamp and Kyriazakis, 1997).*



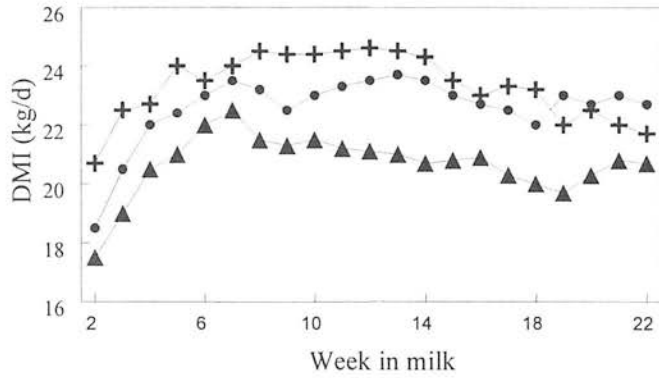
It was therefore concluded that the observed diet selection made nutritional sense in terms of the theory of diet selection described in section 1.2. However, there was no significant correlation between individual diet selection and milk protein output (Tolkamp *et al.*, 1996). To see if ruminants, like non-ruminants, were able to select a diet that reflected their changes in their (protein) internal state through-out lactation, the experiment was repeated over a forty week period using cows that had different genetic merit for milk yield (Tolkamp *et al.*, 1998a).

As with the previous experiment (Tolkamp and Kyriazakis, 1997), two foods, both of which were a mixture of grass silage and concentrates (concentrate: silage ratio was 58:42 on a DM basis), differing in their MP yield (HP, 114g MP/kg and LP, 75g MP/kg) were offered as a choice. Again, cows offered the choice of foods attained food intakes and milk yields similar to the control group offered the HP food, whereas the control group offered LP alone did not (Figure, 1.5 a, b). The average proportion of the HP selected was 687± 5g HP/kg intake which differed significantly ($p<0.001$) from random. However, in agreement with the previous experiment, neither stage of lactation nor milk protein output affected diet selection. The calculated MP intakes of the choice group were higher than the estimated requirements (Figure 1.5 c). This over-consumption of MP was consistent with results from pregnant sheep (Cooper *et al.*, 1994) and lactating cows (Lawson *et al.*, 2000) offered two foods identical in ME yield but differing in the MP yields.

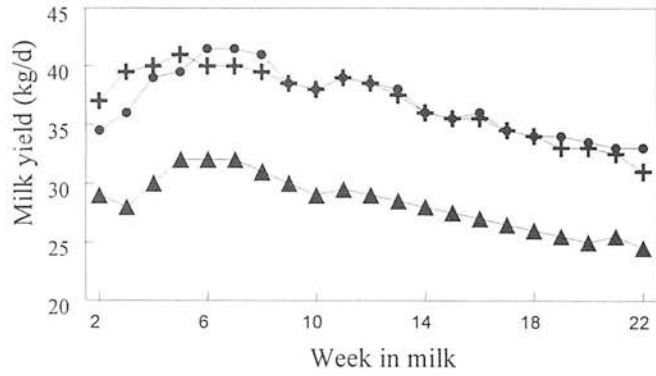
Due to the absence of effects of milk protein output on diet selection, Tolkamp *et al.*, (1998b) proposed a hypothesis as a possible explanation for the observed behaviour. It was hypothesised that cows may have selected for food characteristics other than MP yield. The observed feeding behaviour may have been an attempt by the cows to optimise the supply of eRDP. As the supply of amino acids comes largely from microbial protein, provided that the diet has sufficient eRDP (Van Soest, 1994), the idea that ruminants will select a diet that meets the requirement for eRDP is not therefore an unreasonable one. Should ruminants select a diet to meet the requirements for eRDP, then the excess consumption of MP may be a consequence of this selection, i.e. due to DUP content of the foods selected.

Figure 1.5 *Group means from (a) dry matter intake (DMI; kg/d),(b) milk yield (kg/d), and (c) metabolisable protein intake minus estimated requirements for maintenance (AFRC, 1993) and milk yield (g/d) in relation to week in milk for cows fed a low protein* food (▲) *or a high protein food* (✚) *and for those cows with access to both the low and high MP foods* (●).

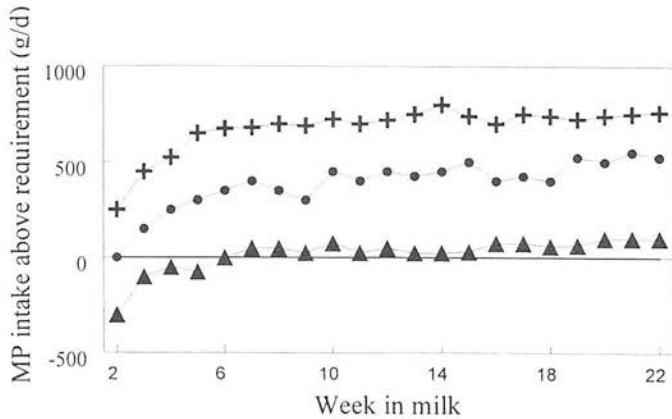
(a)



(b)

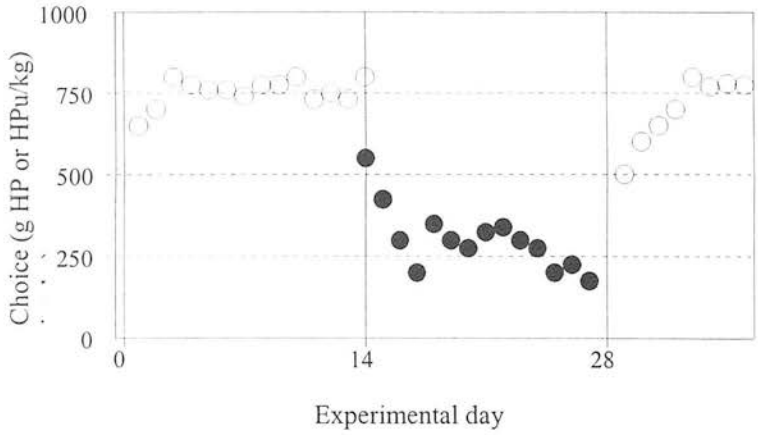


(c)



A further experiment was therefore conducted to measure the effect of adding urea, an RDP source, to both foods LP and HP offered as a choice to determine if altering the eRDP content of the foods would alter diet selection (Tolkamp *et al.*, 1998b). Figure 1.6 shows the results. From the first day after urea was added to both foods, the preference for the HP food was reduced significantly. The proportion of HP food selected changed from 761(S.E. 20)g HP/ kg total food intake (TFI) to 169 (S.E. 22)g HP/ kg TFI. The reverse occurred when the urea was removed; the proportion of HP food selected increased to 726 (S.E. 42)g HP/kg TFI. There were no effects on milk yield or food intake. It was therefore concluded that urea, an RDP source, had a strong effect on diet selection.

Figure 1.6 *Effects on diet selection of adding urea to both the high- and low- protein foods. Diet selection is expressed as g of HP (○) or g of HP with urea (●) per kg intake (Tolkamp et al., 1998b).*



Calculations on the estimated eRDP: fME ratio selected indicated that a ratio of 11g eRDP: 1MJ fME was selected. This was in agreement with the recommended ratio required to optimise microbial protein synthesis (AFRC, 1993). The addition of urea to the LP and HP foods still permitted a wide range MP to ME, as well as eRDP to fME, ratios to be selected. When the urea supplemented foods were offered the diet selected had a lower ratio of MP to ME than when the urea was absent. Therefore, the differences in choice between feeds containing and not containing urea were not

interpreted as an attempt to maintain MP supply. These results were viewed as evidence of behaviour that ruminants aim to regulate eRDP intake.

These results do lead us to the idea of trade-offs in diet selection. The outcome of at least some of the previous experiments can be seen as the animal needing to trade-off between selecting a diet to meet its eRDP requirements, and one that avoids an excess intake of MP. The costs associated with the consumption of excess MP were given little weighting by the animal compared with the benefits of meeting the eRDP requirements (Kyriazakis *et al.*, 1999). The diet selected can therefore be considered as the outcome of the weighting that is given to the relevant benefits and costs.

However, the foods offered as a choice in this experiment differed in characteristics other than their eRDP content, and this did include the MP yield although it was suggested that the results could not be interpreted in terms of MP. The DUP content of the foods and the food ingredients also differed between the two foods offered as a choice. Factors other than eRDP may thus have influenced the diets selected. Therefore, there is a need to investigate further the hypothesis that diets selected by ruminants would be those that meet the eRDP requirements of the animals and at the same time avoid excess consumption of RDP.

1.5 Attributes of the rumen environment that are relevant to food intake and diet selection.

In the previous section the idea of trade-off was introduced in the diet selection of ruminants. Ruminants appeared to select a diet that met their eRDP requirements even if this meant that excess MP was consumed. Another example in which an animal may have to make a trade-off between the foods offered is when it has the opportunity to correct, through its diet selection, imbalances in its internal state, such as a low rumen pH, that has been created by, for example, the consumption of a highly fermentable, high energy food. That is, ruminants will seek to return as soon as possible to an appropriate state.

Ruminants may modify their diet selection in an attempt to prevent 'imbalances' such as a reduction in rumen pH or in an attempt to correct such imbalances should they have already occurred. Such a dietary strategy would bring indirect benefits, namely high rates of microbial growth (Hespell and Brant, 1979) and subsequently, nutrients to the host ruminant. Therefore it has been suggested that one of the objectives of the diet selection of ruminant animals might be to maintain the rumen environment within a desired physiological range (Cooper *et al.*, 1995, 1996; Faverdin, 1999). This section will describe the attributes of the rumen environment that affect food intake and diet selection.

1.5.1 *The rumen and the rumen environment*

The rumen provides a reservoir wherein food, saliva and other secretions are mixed in the presence of micro-organisms which ferment the consumed food (Hungate, 1966; Webster, 1993). The size of the rumen allows food to accumulate and ensures that sufficient time is allowed for the slow breakdown of food components such as the structural plant carbohydrates (Ørskov and Ryle, 1990). Fermentation of the food yields principally VFAs, microbial cells and the gases methane and carbon dioxide (Leng and Nolan, 1984). The gases are lost via eructation and the VFAs are mainly absorbed through the rumen wall (Dijkstra, 1994). The microbial cells, along with undegraded food components, pass to the abomasum and small intestine where the host's enzymes digest them and the products of digestion absorbed (Van Soest, 1994).

The rumen has a number of homeostatic mechanisms. The acids produced by fermentation are theoretically capable of reducing the rumen pH to below 4.5 subject to no maintenance of pH (Hill and Holman, 1989). Under 'normal' conditions, the pH is maintained between 6 and 6.5 by the rumen buffering system (Counette *et al.*, 1979). This system includes phosphates and bicarbonate in the saliva (Turner and Hodgett, 1955; McManus, 1959; Ørskov, 1992) and rapid absorption of the acids (Ash and Dobson, 1963; Dijkstra *et al.*, 1993; Dijkstra, 1994) and the foods buffering

capacity (McBurney *et al.*, 1983; Jasaitis *et al.*, 1987), all of which help to stabilise the pH at a high level.

However, in some cases, especially when readily fermentable foods are consumed (Kaufman, 1976; Baik *et al.*, 1997), the regulation of the rumen pH fails and the pH falls. This can have detrimental effects on both the resident micro-organisms and the animals health and productivity (Russell and Dombrowski, 1980; Owens *et al.*, 1998).

Different micro-organisms tolerate different ranges of pH. The pH of the rumen environment determines which micro-organisms predominate in the rumen ecosystem. As the pH falls, there is a fall in cellulolytic bacteria activity (Russell *et al.*, 1979; Mould and Ørskov, 1984). This has been shown to rapidly reduce food intake due to cellulose fermentation being abolished (Mould and Ørskov, 1984). Amylolytic bacteria still manage to multiply and ferment nutrients at the lower pH (Russel and Dombrowski, 1980), but at a reduced rate. Therefore, as the pH decreases the overall fermentation process declines (Erfle *et al.*, 1982).

The decrease in VFA production, in conjunction with energy being used to maintain bacterial cells as the pH of the rumen falls (Russel and Hespell, 1981), has a direct effect on the host as less ME is available per unit food consumed. In addition, reduced microbial activity, and hence protein synthesis, results in a decrease in the microbial protein yield (Mould and Ørskov, 1984; Goad *et al.*, 1998). On top of this, further reductions in the rumen pH can result in ruminal, and subsequently metabolic, acidosis that can prove fatal (Owens *et al.*, 1998). Even if the ruminant recovers from a bout of acidosis, nutrient absorption may be reduced and performance depressed. Therefore it would be advantageous for ruminants to modify their feeding behaviour to prevent such declines in rumen pH as this would reduce the fall in microbial activity hence maintain the nutrient supply to the host, and help prevent the occurrence of acidosis. Modifications in the feeding behaviour of ruminants to prevent such declines in the rumen pH has been observed by Cooper *et al.* (1995, 1996) and Phy and Provenza (1998 a, b) under experimental conditions.

1.5.2 *The effect of rumen pH on food intake and diet selection*

Considerable evidence exists that indicates that the conditions within the rumen can influence food intake and diet selection (Williams *et al.*, 1987). For example, Bhattacharya and Warner (1967) demonstrated that intraruminal infusion of phosphoric, lactic and citric acid resulted in a significant decreases in the intake of hay. This may be due to the reduced microbial activity, and hence reduced fermentation (Russell and Dombrowski, 1980; Erfle *et al.*, 1982; Mould and Ørskov, 1984), or a reduction in rumen motility (Dougherty *et al.*, 1975; Crichlow, 1988) due to the decrease in rumen pH. As the results indicate that a drop in rumen pH result in a decline in food intake, this could be of physiological importance with some foods, especially readily fermentable foods.

The mechanisms of the effects of pH on intake are uncertain. It is difficult to manipulate the pH of the rumen without altering other aspects of the rumen environment such as osmolality (Bhattacharya and Warner, 1967) and rumen motility (Dougherty *et al.*, 1975; Crichlow, 1988). However it is generally accepted (Counette *et al.*, 1979; Forbes and Barrio, 1992) that rapid ingestion of readily fermentable carbohydrates results in a decrease in rumen pH which is frequently associated with a reduction in food intake (Phy and Provenza, 1998a). The fact that the readily fermentable carbohydrates ingested, and hence a fall in the rumen pH, results in a reduction in food intake suggests that these factors may also modify diet selection when the ruminant has a choice of foods that have different effects on the rumen environment (Kyriazakis *et al.*, 1999).

Cropper (1987) speculated that ruminants select a diet that 'enables its rumen to remain in an adaptive state'. This suggestion was made to account for the dietary choices made by sheep that selected a mixture of the two foods offered, that were of high and low digestibility, rather than predominantly choosing the higher digestible food as has been demonstrated in non-ruminants (Ferguson *et al.*, 1998, 1999). The selection of a mixed diet has been frequently shown in grazing ruminants when both higher and lower digestible foods are available (Newman *et al.*, 1994; Parsons *et al.*,

1994; Cooper *et al.*, 1995, 1996; Concha and Nicol, 2000). The potential disruptive effects on the rumen environment, such as a fall in pH caused by the consumption of a high ED, readily fermentable food (Kennelly *et al.*, 1999; Hristov *et al.*, 2001) may explain why ruminants do not select a diet that consists predominantly of the more digestible, higher energy food. Cooper *et al.* (1995) looked at the relationship between the rumen environment and the diet selected by sheep from foods of different energy density. Sheep were offered a choice between a high and low energy density food and although the rate of live weight gain was greatest on the higher energy density food, sheep selected 320g/kg food intake as the lower energy density food. Both the decrease in food intake, and the modification in the diet selected by ruminants offered foods that disrupt the rumen environment, are in agreement with the hypothesis that ruminants alter their feeding behaviour to promote effective rumen conditions (Cooper *et al.*, 1995, 1996; Faverdin, 1999). This would aid the animal in achieving subsequent food intake that allows nutrient and energy requirements to be met.

It could therefore be hypothesised that ruminants include a significant proportion of the low energy density food in their diet to dilute the disruptive effects of the high energy density food when they are given a choice. However, the incorporation of a lower energy density food into the diet could result in an inability to meet energy requirements, compared to when the diet consists solely of the high ED food (Kyriazakis and Oldham, 1993; Cooper *et al.*, 1995). Therefore, ruminants could be seen as facing a trade-off between the potential benefits of selecting a higher energy food against the potential cost of disrupting the rumen environment, when selecting their diet from two foods that have different effects on the rumen environment (Kyriazakis *et al.*, 1999).

Earlier, the idea of trade-off was applied to ruminant animals where these animals appeared to select a diet that met their eRDP requirements even if this meant that excess MP was consumed. The diets selected by animals that attempted to obtain their requirements for eRDP and those that attempted to maintain their rumen environment within a desired physiological range, were the outcome of the

weightings given to the relevant benefits and costs.

However, it is highly unlikely that ruminants will try to keep their rumen environment at a constant state, where small deviations in the rumen environment warrant modifications in the diet selected to correct the deviations as suggested by Provenza *et al.* (1998). More likely there will be a range of conditions that the animal will tolerate (e.g. Cooper *et al.*, 1995; Kyriazakis & Oldham, 1997). Therefore, should changes to the rumen environment exceed these 'tolerance' limits, by, for example, the consumption of a highly disruptive, readily fermentable concentrate food, modification to the diet selected could be expected (Engku Azahan & Forbes, 1992; Mbanya *et al.*, 1993). Therefore, a large, rather than small, change in the rumen environment would be expected to alter subsequent diet selection (Kyriazakis *et al.*, 1999). These hypotheses constitute the subject of experiments addressed in this thesis.

1.6 Summary

The expectation of the diet selection for protein is that animals will select a diet that both meets their protein requirements and avoids excess when offered an appropriate choice. From this review of literature, the evidence from non-ruminant animals suggests that non-ruminants are able to select a diet that reflects their protein requirement, whilst avoiding an excess consumption of protein, when offered an 'appropriate' choice of foods and sufficient time is allowed for the animals to learn about the foods offered as a choice. However, the results from experiments with ruminants have not always been consistent with this view and this inconsistency may reflect the greater complexity of the ruminant digestive system in dealing with protein.

An alternative hypothesis suggested to account for these apparently conflicting findings was that ruminants might select their diet on the basis of eRDP content rather than MP yield. The amino acid supply for ruminants comes largely from

microbial protein, provided that the diet has sufficient eRDP. Therefore, the idea that ruminants will select a diet that meets the requirement for eRDP, which is captured and utilised by the microbes to produce microbial protein, is not therefore an unreasonable one. However, results to date are far from conclusive resulting in a need to investigate further the hypothesis that diets selected by ruminants would be those that meet the eRDP requirements of the animals and at the same time avoid excess consumption of RDP.

The above hypothesis does lead us to the idea of trade-offs in diet selection. The outcome of at least some of the previous experiments with ruminants described in Section 1.4.4 can be seen as the ruminant needing to trade-off between selecting a diet to meet its eRDP requirements, and one that avoids an excess intake of MP. The costs associated with the consumption of excess MP were given little weighting by the animal compared with the benefits of meeting the eRDP requirement. The diet selected can therefore be considered as the outcome of the weighting that is given to the relevant benefits and costs.

Another example where an animal might make a trade-off between the foods offered is when it has the opportunity to correct, through its diet selection, imbalances in its internal state that have been created. That is, an animal will seek to return as soon as possible to an appropriate state.

Such an imbalance in the ruminant's internal state may be the reduction in the pH of the rumen environment caused by the consumption of a rapidly fermentable, high energy food. Ruminants would be expected to alter their feeding behaviour to maintain the pH of the rumen environment within a desired physiological range. This in turn would aid the animal in achieving subsequent food intake that allows nutrient and energy requirements to be met. It could therefore be hypothesised that ruminants include a significant proportion of the low energy density food in their diet to dilute the disruptive effects of the high energy density food when they are given a choice. However, the incorporation of a lower energy density food into the diet could result in an inability of the ruminant to meet energy requirements, compared to when the

diet consists solely of the high energy density food. Therefore, ruminants could be seen as facing a trade-off between the potential benefits of selecting a higher energy food against the potential cost of disrupting the rumen environment, when selecting their diet.

1.7 Aim of thesis

The aim of this thesis was to investigate the effects of rapidly degradable nitrogen and rapidly fermentable material within the rumen on the diet selection of ruminants.

The objectives of the thesis were to test the hypotheses that:

1. Ruminants will select a diet to obtain an estimated 'optimal' eRDP: fME ratio of 10:1 from two foods that differ mainly in their eRDP content (Chapter 2).

2. Ruminants will avoid both deficient and excessive eRDP levels with respect to the fME content of the food when offered a choice (Chapters 3 and 4).

3. The desire of the ruminant to maintain the rumen within a desired physiological range will override other needs, such as the avoidance of an excess intake of RDP (Chapter 4).

4. Ruminants select a diet as a result of a trade-off between the potential benefits of selecting a higher energy food against the potential cost of disrupting the rumen environment, when selecting their diet from two foods that have different effects on the rumen environment (Chapter 5).

5. Changes within the rumen of 'large' rather than 'small' magnitude, due to the consumption of a disruptive food, are required to alter subsequent diet selection by sheep (Chapters 4 and 5).

Chapter 2

Do sheep select a diet to achieve an estimated 'optimal' eRDP: fME ratio?

Advances are made by answering questions. Discoveries are made by questioning answers.
Bernhard Haisch

2.1 Abstract

The hypothesis tested was that growing sheep would select a diet to obtain an estimated 'optimal' effective rumen degradable protein (eRDP) to fermentable metabolisable energy (fME) ratio of 10:1, from two foods that differ mainly in their eRDP content. The fME content of both foods of the pair was either low or high. Two basal foods H and L, which differed mainly in energy density, were formulated. Food H was designed to at least meet the fME (9.6 MJ ME/kg fresh matter (FM)) and metabolisable energy (ME; 10.5 MJ ME/kg FM) requirement for potential growth of sheep of 35kg live weight. Food L was designed to be deficient in fME (7.2 MJ ME/kg FM) and ME (8 MJ ME/kg FM) to support potential growth when fed alone on an *ad libitum* basis. Both foods had similar calculated metabolisable protein (MP) yield (mean 48.8g MP/kg FM) and eRDP (mean 57.4g eRDP/kg FM) content, but were deficient in MP and eRDP to support potential growth when fed alone on an *ad libitum* basis. Six additional foods were formulated by adding 7.5, 15 and 22.5g urea/kg FM to both H and L. The experiment consisted of eight, 4x4 Latin squares with four treatments and four periods, each period lasting for two weeks. Thirty-two, Texel x Greyface sheep were randomly allocated to one of the eight Latin squares (n=4). Each sheep was offered a choice between two foods. The choices offered were always between foods of the same fME content. For each sheep, one of the foods, offered as a choice, remained the same throughout all four periods of the Latin square. Each food was used as the constant food to create the 8 Latin squares. The urea content (0, 7.5, 15 or 22.5g urea/kg) of the other food offered as a choice changed with each period. The chemical composition of the foods changed throughout the course of the experiment and it was thought this was due to the food ingredients separating. Due to this, the eRDP content of the diet selected by sheep offered a choice could not be satisfactorily predicted. However, when the fME content of both foods was limiting (food L), sheep reduced the proportion of the food with the greater urea-supplementation of the pair offered, selecting an average 0.385g/kg total food intake (TFI) of the higher urea-supplemented food. When the fME content of the food was high (food H), sheep selected a significantly greater ($p<0.01$; s.e.d. 0.1083) proportion of the higher urea-supplementation food of the pair offered, selecting on average 0.513g/kg TFI as the higher urea-supplemented food. The results were in the same direction as that suggested by the hypothesis, but were far from conclusive due to the complications in the actual chemical composition of the foods offered as a choice.

2.2 Introduction

Ruminants derive their protein supply from two sources. One source is from digestible undegraded food protein (DUP), which is the food protein that has not been degraded during its passage through the rumen, but which is sufficiently digestible to be absorbed in the lower intestines of the animal. The other, and in many cases the most important, protein source is microbial crude protein (MCP), which is the protein yielded from the digestion of microbial cells, washed out from the rumen, within the lower intestines of the animal (Agricultural and Food Research Council (AFRC), 1993).

The micro-organisms in the rumen are highly proteolytic, and thus ensure that most of the protein entering the rumen from foods consumed is degraded to yield ammonia via intermediates which include peptides and amino acids (Van Soest, 1994). The ammonia produced, together with some small peptides and free amino acids, is utilised by the rumen micro-organisms to synthesis microbial protein (Russell *et al.*, 1983). As a consequence, it is not surprising that in many instances the majority of the protein that is absorbed from the digestive tract of ruminants is of microbial origin (Nolan and Leng, 1972; Dijkstra *et al.*, 1998). In view of the importance of microbial protein in the supply of protein to the ruminant host, it has been hypothesised that ruminants might select their diet on the basis of effective rumen degradable protein (Tolkamp *et al.*, 1998b). Effective rumen degradable protein (eRDP) has been defined as a measure of the total nitrogen supply that is actually captured and utilised by the rumen microbes for growth (AFRC, 1993). It has been predicted that eRDP is used with an efficiency of 1.0 for microbial protein synthesis.

However, the yield of microbial protein that becomes available for digestion and absorption by the host, has been related to the fermentable metabolisable energy (fME) content of the food consumed (Russell *et al.*, 1983). Accordingly the rumen MCP yield is expressed as g MCP/ MJ fME in the diet (AFRC, 1993). The yield of MCP/ MJ fME in the diet is influenced by the outflow rate of the rumen contents, with an increase in outflow rate increasing the efficiency of microbial protein synthesis (Meng *et al.*, 2000). It has therefore

been estimated that for maximal protein synthesis, rumen micro-organisms of growing sheep, which are considered to have a feeding level between one and two times maintenance, require 1 MJ of fME to capture and utilise 10g eRDP and hence synthesis 10g MCP (ARFC, 1993).

Nitrogen is required by the micro-organisms for maintenance and growth (Al-Rabbat *et al.*, 1972; Orskov, 1992). Should the eRDP content of the food be deficient with respect to the fME content (i.e. below 10g eRDP/ MJ fME for growing sheep) of the food, a reduction in microbial activity could be expected (Kanjanapruthipong and Leng, 1998; Carro and Miller, 1999). Subsequently, MCP yield and the rate of fermentation of ingested food will be reduced and food intake can be expected to fall (Orskov *et al.*, 1971; Ternouth *et al.*, 1993). However, eRDP, and subsequently ammonia, supplied in quantities greater than that required for microbial growth can be absorbed across the rumen epithelium and either returned to the rumen or excreted in the form of urea, incurring energy costs to the host animals (Lobley *et al.*, 1995). Therefore it could be hypothesised that ruminants would be expected to select a diet to obtain an 'optimal' eRDP: fME ratio to maximise microbial protein synthesis, and minimise the costs associated with removal of excess ammonia.

The objective of this experiment was to test the hypothesis that both deficient and excessive eRDP levels in the foods offered as a choice, with respect to the fME content of the food, will be avoided. Urea was used as the additional eRDP source in foods in order to have no appreciable effect on the ingredient composition of the foods used. By using urea there was no distinction made between the eRDP and RDP scale. While eRDP is the relevant scale for requirements RDP would be the relevant scale for excess. The choices given were between foods of different urea contents, but of the same fME contents. It was predicted that irrespective of the fME content of the foods (i.e. high or low fME content), growing sheep would select between the two foods differing in their eRDP content to obtain an optimum ratio of eRDP to fME of 10:1 and therefore enhance microbial protein output from the rumen.

2.3 Materials and Methods

2.3.1 *Animals and Housing*

Thirty-two Texel x Greyface female sheep, approximately four months of age and weighing 34.5(S.D. 3.32) kg at the start of the experiment were used. They were housed in individual pens (2 x 1.5m) on sawdust, in a shed that was naturally ventilated. Natural light was the only source of light used through out the experiment that took place from July to November 1998 at latitude 56°N. The sheep were given 21 days acclimatisation to allow them to become accustomed to their new environment and experimental procedures before the start of the experiment. During this period they were offered *ad libitum*, a high quality pelleted food with 171g crude protein (CP)/kg dry matter (DM) and 11.4 MJ metabolisable energy (ME)/kg DM with no access to long forage. All sheep had free and continuous access to water throughout.

2.3.2 *Experimental foods*

Two basal foods H (high) and L (low), that differed mainly in energy density were formulated (Table 2.1). The foods were presented in a meal form to prevent any changes in the chemical composition of the foods that may occur during the pelleting process (Van Soest, 1982). Food H was designed to at least meet the ME and fME requirements for potential growth of sheep of 35kg live weight (AFRC, 1993). Food L was designed to be deficient in ME and fME to support potential growth when fed alone on an *ad libitum* basis. Both foods had a similar calculated MP yield and eRDP content, but were both designed to be deficient in MP and eRDP to support potential growth when fed alone on an *ad libitum* basis (AFRC, 1993).

Both foods were formulated to be deficient in eRDP and MP so that a range of foods with varying eRDP contents, but with the same fME contents, could be formulated through the addition of urea, an eRDP source (see below).

Table 2.1 *Ingredients and estimated chemical analyses of the two basal experimental foods. The calculated yields of protein and ME components are also given.*

	Foods	
	H	D
Ingredients (g/kg)		
Barley	420	340
Oatfeed	140	551
Citrus Pulp	400	0
Soya Bean Meal (solvent extracted)	0	60
50% Fat Premix†	25	25
Salt	5.4	5.3
Dicalcium Phosphate	7.9	6.3
Limestone Flour	0	9.6
Calcified Magnesite	0	1.1
Vitamin and Mineral Mix‡	1.7	1.7
Total	1000	1000
Chemical Analysis (g/kg DM)		
Dry Matter (g/kg)	882	891
Crude Protein	87	91
Crude Fibre	121	176
Modified Acid Detergent Fibre	186	256
Neutral Detergent Fibre	298	469
Ash	60	67
Calcium	7.8	7.9
Phosphorus	4.0	4.0
Sodium	2.6	2.6
Sulphur	1.1	1.4
Estimated yields§		
Metabolisable Energy (ME; MJ/kg DM)‖	11.9	9.0
Fermentable ME (fME; MJ/kg DM)	10.9	8.1
Metabolisable Protein (MP; g/kg DM)	53.9	56.2
eRDP (g/kg DM)¶	64.4	65.1
Digestible undegraded protein (g/kg DM)		
MP:ME (g/MJ)	4.5	6.2
eRDP:fME (g/MJ)	5.9	8.0

†, Manufactured and supplied by Central Farmers Ltd, Methil, Fife, Scotland. ‡, Vitamin and Mineral mix used was Scotmin ewe/lamb (Scotmin Nutrition Ltd, Ayr, Scotland). §, Values calculated using the metabolisable protein system (AFRC, 1993) assuming rumen outflow rate of $0.05h^{-1}$ and standard values for degradability coefficients (MAFF, 1990). ‖ , ME, calculated using the following equation (ME = 0.14(Neutral cellulase gaminase digestibility) + 0.25(Acid Hydrolysed ether extract); Thomas *et al.*, (1988)). ¶, eRDP, effective rumen degradable protein.

Six additional foods were formulated by adding 7.5, 15 and 22.5g urea/kg fresh matter to H (H series; H, $H_{7.5}$, H_{15} and $H_{22.5}$ respectively) and L (L series; L, $L_{7.5}$, L_{15} and $L_{22.5}$ respectively). Urea was used as a source of eRDP because it allows a large change in the eRDP content of the food with no appreciable effects on the ingredient composition. The concentrations of urea selected were such that allowed us to demonstrate whether sheep selected a diet that avoided both deficient and excessive amounts of eRDP with respect to the fME content of the foods (see below).

Table 2.2 *The calculated effective rumen degradable protein (eRDP) and calculated metabolisable protein (MP) contents and the eRDP: fermentable metabolisable energy (fME) ratios of the two basal foods, without and with the addition of 7.5, 15 or 22.5g urea/kg fresh matter.*

Basal Food	Urea added (g/kg fresh matter)	Calculated eRDP content (g/kg DM)	Calculated MP content (g/kg DM)	eRDP:fME ratio (g/MJ)	MP:ME ratio (g/MJ)
H	0	64.4	53.9	5.92	4.5
	7.5	84.2	66.6	7.75	5.59
	15	104.0	79.2	9.57	6.65
	22.5	123.8	82.2	11.39	6.90
L	0	65.1	56.2	8.08	6.24
	7.5	84.7	66.0	10.51	7.33
	15	104.3	66.0	12.94	7.33
	22.5	123.9	66.0	15.37	7.33

In addition, these urea concentrations would not be expected to result in a random diet selection (50:50), if sheep were to select a diet for an estimated 'optimal' eRDP: fME ratio of 10:1. The calculated eRDP and MP contents and the eRDP: fME and MP: ME ratios of the two basal foods, without and with the addition of 7.5, 15 or 22.5g urea/kg fresh matter are shown in Table 2.2.

2.3.3 *Experimental procedure*

The experiment consisted of eight, 4x4 Latin squares with four treatments and four periods, each period lasting for two weeks. The sheep were randomly allocated to one of the eight Latin squares (n=4). Each sheep was offered a choice between two foods. The choices offered were always between foods with the same fME content. For each sheep, one of the foods offered as a choice remained the same throughout all four periods of the Latin square. The urea content (0, 7.5, 15 or 22.5g urea/kg) of the other food offered as a choice changed with each period (Table 2.3). The order of the second food offered within a choice was balanced across sheep within a square to account for any residual effects.

Table 2.3 *The concentration of urea added (g urea/kg fresh matter) to the foods offered as a choice during each of the 4 periods of the Latin square. Both foods offered as a choice had the same fME content. In the example shown here, the unsupplemented basal food (0) is the food that remains constant through out the experiment.*

Period	Sheep			
	1	2	3	4
1	0 vs. 0	0 vs. 7.5	0 vs. 22.5	0 vs.15
2	0 vs. 7.5	0 vs. 22.5	0 vs. 15	0 vs.0
3	0 vs. 15	0 vs. 0	0 vs. 7.5	0 vs. 22.5
4	0 vs. 22.5	0 vs. 15	0 vs. 0	0 vs. 7.5

For example, sheep offered food H as the constant food received H vs. H, H vs. $H_{7.5}$, H vs. H_{15} and H vs. $H_{22.5}$ choices for periods one to four of the Latin square respectively. This design provides one period where the two foods offered as a choice are the same, which will allow the measurement of food intake and performance on each single food to be taken as a control. Each Latin square was repeated with $H_{7.5}$, H_{15} and $H_{22.5}$ as the constant foods. This design was repeated for the L basal foods to create the eight Latin

squares. The position of the food bins was randomised across all sheep and the position did not change through out the experiment.

2.3.4 *Measurements*

Fresh food was weighed and offered at 0830 hours each day and refusals were weighed and discarded. The live weights of the sheep were measured just prior to feeding on the first day of the experiment and weekly thereafter.

2.3.5 *Food analysis and digestibility trial*

Samples of all foods were taken prior to, and at the end of the experiment, and were subsequently analysed for DM, ash, neutral detergent (plus amylase) fibre (NDF), acid detergent fibre (ADF), acid hydrolysed ether extract, neutral cellulase gaminase digestibility (NCGD), sodium, calcium and phosphorous contents (Table 2.1) and CP and urea (Table 2.4) as described by Ministry of Agriculture, Fisheries and Food ((MAFF), 1993).

The DM digestibility of all 8 foods used in this experiment was measured on 16 sheep selected from the diet selection experiment. The digestibility trial ran for four weeks immediately after the diet selection experiment. During this trial sheep were only offered foods of which they had previous experience. Therefore 8 sheep selected for the digestibility trial had previously been offered a choice of foods from the H series, whilst the other 8 sheep had been offered a choice of foods from the L series. Sheep from the H series were randomly allocated one of H, $H_{7.5}$, H_{15} or $H_{22.5}$ (n=2). Sheep from the L series were randomly allocated to one of L, $L_{7.5}$, L_{15} or $L_{22.5}$ (n=2).

All sheep were offered their allocated food for a period of one week whilst housed in individual pens. On day eight of this trial all sheep were moved to metabolic crates where they continued to receive their allocated food *ad libitum*. On day fifteen, sheep

were moved back to their individual pens and offered another food, again with the same fME content but with a different urea and hence eRDP content, to that of the previous two weeks. The two sheep that were offered the same food during days 1 to 14 were offered different foods on days 15 to 28. On day 22 all sheep were moved back to the metabolic crates for a further week where they continued to receive the second allocated food *ad libitum*.

Fresh food was weighed and offered at 0830 hours each day and refusals were weighed and discarded. Samples of food refusals were collected for subsequent DM analysis. All faeces excreted were collected daily, weighed and frozen for subsequent DM analysis. The DM digestibility of each food was calculated by dividing the total DM intake minus the total DM in the faeces by the total DM intake.

2.3.6 *Statistical Analysis*

All data were analysed using GENSTAT for Windows (release 3.2, Lawes Agricultural Trust, 1993) unless otherwise stated.

2.3.6.1 *Digestibility trial.*

The food intake data during the period when sheep were in the metabolism crates and the food digestibility data were analysed initially as a 2x2x4 factorial design (2 periods; x 2 basal foods L and H; x4 levels of urea supplementation 0, 7.5, 15 and 22.5g urea/kg) using ANOVA. Effects of and interactions between period, basal foods and urea supplementation were initially analysed with period, basal food and urea supplementation as factors. As period was found not to have a significant effect, the data were re-analysed as a 2x4 factorial design with basal food and urea supplementation as factors.

2.3.6.2 *Diet selection experiment.*

Using ANOVA, data from choice fed sheep for daily food intake and diet selection, expressed as the proportion of total food taken as the food that remained constant through out the 8 week period, were initially analysed for each Latin square separately. The data for food intake and the diet selected from the second week of each period were used for the statistical analysis. Data from the first week of each period were excluded as it was expected that this period represented the time that sheep would learn about the foods (Forbes and Kyriazakis, 1995; Tolkamp and Kyriazakis, 1997). In all analysis of data from the choice fed sheep, period was nested within sheep as a block factor to take into account the fact that the same sheep were used in all periods. The food intake and diet selection data were analysed for the effects of, and interactions between, period and urea choice (see Materials and Methods for choices offered), with these factors as treatments.

Period was found not to have any significant effect. In addition, due to the unexpected results from the food analysis results (see below), the data from the diet selection experiment were pooled across all Latin squares. Data from sheep offered the same two foods as a choice (e.g. 0 vs. 0, 7.5 vs. 7.5) were analysed separately using ANOVA to investigate the effects of, and interactions between, basal food (L and H) and urea supplementation (0, 7.5, 15 and 22.5g urea/kg) on food intake, using basal food and urea supplementation as factors.

Using ANOVA, the remaining pooled data from the choice fed sheep for daily food intake and diet selection were analysed for the effects of, and interactions between, basal food (L and H) and choice (0 vs. 7.5, 0 vs. 15, 0 vs. 22.5, 7.5 vs. 15, 7.5 vs. 22.5 and 15 vs. 22.5 g urea/kg) with basal food and choice as factors. To test whether the proportion of food selected differed from random (0.5) a Student's 't'-test with a null hypothesis of mean = 0.5 for each group was used (Minitab for Windows release 11.1, Minitab Inc., 1996).

For each sheep the linear regression coefficient for weight on time, over each two week choice period were used to estimate live weight gain. The rates of gain data were analysed in the same way as daily food intake.

2.4 Results

2.4.1 *Food analysis*

The expected, and measured, CP and urea contents of the eight experimental foods used in this experiment are given in Table 2.4. The CP contents of the basal foods prior to urea supplementation were higher in the samples taken at the start of the experiment than originally expected. In addition the amount of urea measured from the samples collected at the start of the experiment did not always correspond to the supplement amounts, particularly in the L series. For this series the CP and urea contents did systematically increase as required for the experiment, though not in the required amounts.

Table 2.4 *The expected, and measured at the start and end of the experiment, crude protein (g CP/kg fresh matter (FM)) and urea (g urea/kg FM) content of the eight experimental foods.*

Food	Expected (g/kg FM)		Start (g/kg FM)		Finish (g/kg FM)	
	CP	Urea	CP	Urea	CP	Urea
H	77	0	105	0.6	103	0.8
$H_{7.5}$	98	7.5	121	7.7	106	6.5
H_{15}	120	15	157	19.5	99	9.0
$H_{22.5}$	142	22.5	149	25.3	125	14.4
L	81	0	103	0.7	81	0.7
$L_{7.5}$	104	7.5	117	7.5	107	6.8
L_{15}	126	15	130	9.8	112	8.2
$L_{22.5}$	147	22.5	138	16.6	128	14.2

However, the urea content of the foods in the H series also systematically increased, but the CP content of $H_{22.5}$ was lower than that measured for H_{15}.

As can be seen from Table 2.4, the CP contents of the foods measured at the end of the experiment differed from those collected from samples at the beginning of the experiment with the CP content being similar in all foods except those supplemented with 22.5g urea/kg. Therefore the composition and the eRDP: fME ratio of the diets selected by sheep in this experiment could not be reliably estimated.

2.4.2 *Digestibility trial*

Daily food intake and DM digestibility of the eight experimental foods during the digestibility trial when the sheep were housed in metabolism crates are given in Table 2.5.

Table 2.5 *The dry matter (DM) digestibility (%) and food intake (g/d) of the two basal foods (H and L) and these two foods supplemented with 0, 7.5, 15 and 22.5g urea/kg FM offered* ad libitum *to sheep housed in metabolism crates.*

Urea added (g/kg FM)	DM digestibility (%)		Food Intake (g/d)	
	H series	L series	H series	L series
0	57.1	38.5	1419	1664
7.5	60.9	48.4	1508	1853
15	62.2	45.2	1308	1796
22.5	64.6	44.1	1444	1834
Mean	61.2	44.1	1420	1788
s.e.d	5.96		211.9	
Basal	***		**	
Urea	NS		NS	
Basal*Urea	NS		NS	

Daily food intake was significantly affected by the basal food offered. Sheep offered foods from the L series has significantly ($p<0.01$) greater daily food intakes than sheep offered foods from the H series. Daily food intake was not significantly affected by urea supplementation, nor were there any significant interactions between basal food and urea supplementation.

The DM digestibility was significantly affected by the basal food. The DM digestibility was significantly ($p<0.001$) greater for foods from the H series than for foods from the L series. Urea supplementation did not significantly affect the DM digestibility, nor were there any significant interactions between the basal food and urea supplementation.

2.4.3 *Diet selection experiment*

2.4.3.1 *Sheep offered one food*

The daily food intake of sheep offered two identical foods as a choice, hence used as controls, is given in Table 2.6. Daily food intake was significantly affected by the basal foods offered only.

Table 2.6 *The daily food intake (g/d) by sheep offered basal foods differing in calculated fME content (H contained 9.6 MJ fME/kg FM; L contained 7.2 MJ fME/kg FM) and urea supplementation (0, 7.5, 15 and 22.5 g urea/kg FM).*

Urea content of foods offered:	Intake (g/d)	
	H series	L series
0 vs. 0	1914	2101
7.5 vs. 7.5	1904	2267
15 vs. 15	1903	2010
22.5 vs. 22.5	1789	2202
Mean	1878	2145
s.e.d	128.3	
Basal food	*	
Urea	NS	
Basal*Urea	NS	

Sheep offered foods from the L series had significantly ($p<0.05$) greater daily food intakes than sheep offered foods from the H series. Daily food intake was not significantly affected by urea supplementation, nor were there any significant interactions between basal food and urea supplementation.

Neither basal food, nor urea supplementation had an effect on daily live weight gain. Live weight gain for sheep offered foods from the H or L series were 223 and 246 g/d (s.e.d. 38.9) respectively.

2.4.3.2 *Choice fed sheep.*

The expected proportion of the lower urea-supplemented food selected by sheep offered foods differing in their eRDP content through the addition of urea but not their fME content, assuming that sheep would select a diet to obtain the expected 'optimum' eRDP: fME ration of 10:1, are given in Table 2.7. Table 2.8 shows the actual proportion of the lower urea-supplemented food selected by sheep offered foods differing in their eRDP content but not their fME content.

Due to the problems associated with the results from the food analysis, the results from the actual proportions of the lower urea-supplemented food selected are not expected to correspond to those originally assumed. However, sheep on the H series offered 0 vs. 22.5g urea/kg did show a significant ($p<0.05$) preference for the urea-supplemented food, which is in agreement with the predicted diet selection. The sheep offered the same choice of foods on the L series also showed a significant ($p<0.05$) preference (i.e. different from random selection), but this time for the food not supplemented with urea, which is in the same direction as predicted. Sheep on the L series offered 7.5 vs. 22.5g urea/kg also showed a significant ($p<0.05$) preference for the food with the lower urea content. The diets selected by all other groups did not differ significantly from random. Overall, the basal food to which the urea was supplemented significantly affected the proportion of lower urea food selected. Sheep offered a choice of foods from the L series showed a significantly ($p<0.01$) greater preference for the lower urea supplemented foods compared to sheep offered a choice of foods from the H series (Table 2.8).

Table 2.7 *The expected proportion of the lower urea supplemented food selected by sheep offered foods differing in their eRDP (effective rumen degradable protein) content through the addition of urea but not their fME (fermentable metabolisable energy) content presuming sheep select a diet to obtain the expected 'optimum' eRDP: fME ration of 10:1 The proportion expected to be selected is expressed as g lower urea supplemented food selected/kg total food intake.*

Urea content of foods offered:	Expected proportion selected of the lower urea food (kg/kg)	
	H series	L series
0 vs. 7.5	0.00	0.00
0 vs. 15	0.00	0.60
0 vs. 22.5	0.20	0.75
7.5 vs. 15	0.00	1.00
7.5 vs. 22.5	0.30	1.00
15 vs. 22.5	0.60	1.00

Table 2.8 *The actual proportions of the lower urea supplemented food selected by sheep offered foods differing in their eRDP (effective rumen degradable protein) content through the addition of urea but not their fME (fermentable metabolisable energy) content. Asterisks (*) next to the proportion on lower urea food selected denote that the diet selected differed significantly from random (0.5). The proportion selected is expressed as g lower urea supplemented food selected/kg total food intake.*

Urea content of foods offered:	Proportion of lower urea food selected (kg/kg)	
	H series	L series
0 vs. 7.5	0.489	0.591
0 vs. 15	0.437	0.588
0 vs. 22.5	0.388*	0.770*
7.5 vs. 15	0.505	0.546
7.5 vs. 22.5	0.533	0.687*
15 vs. 22.5	0.528	0.514
Mean	0.478	0.615
s.e.d	0.1083	
Basal	**	
Urea choice	NS	
Basal*Urea	NS	

The daily food intake by sheep offered a choice between two foods that differ in their eRDP content through the addition of urea, but not their fME content, are given in Table 2.9. In agreement with the results from the single fed sheep, daily food intake was significantly affected by the basal foods offered. Sheep offered foods from the L series had significantly ($p<0.001$) greater daily food intakes than sheep offered foods from the H series. Daily food intake was not significantly affected by urea supplementation, nor were there any significant interactions between basal food and urea supplementation.

Table 2.9 *The daily food intake by sheep offered a choice of foods differing in their eRDP (effective rumen degradable protein) content through the addition of urea but not their fME (fermentable metabolisable energy) content.*

Urea content of foods offered:	Food intake (g/d)	
	H series	L series
0 vs. 7.5	1914	2135
0 vs. 15	1869	2095
0 vs. 22.5	1748	2089
7.5 vs. 15	2002	2069
7.5 vs. 22.5	1744	2196
15 vs. 22.5	1718	2107
Mean	1833	2115
s.e.d.	168.8	
Basal food	***	
Urea	NS	
Basal*Urea	NS	

2.5 Discussion

Foods deficient in eRDP, with respect to the fME content of the food, are expected to result in a reduction in microbial activity and subsequently the rate of fermentation of ingested food (Kanjanapruthipong and Leng, 1998; Carro and Miller, 1999). This in turn has been demonstrated to result in a decrease in food intake (Orskov *et al.*, 1971; Ternouth *et al.*, 1993). However, nitrogen supplied in quantities greater than that required for microbial growth can be absorbed across the rumen epithelium and either returned to the rumen or excreted in the form of urea, incurring energy costs to the host animals (Lobley *et al.*, 1995). Therefore it was hypothesised that growing sheep would select a diet, from two foods differing in their eRDP content, to obtain an 'optimal' eRDP: fME ratio of 10:1 and therefore maximise microbial protein synthesis (AFRC, 1993) and minimise costs. The objective of the experiment reported here was to test the hypothesis that both deficient and excessive eRDP levels with respect to the fME content of the food would be avoided. The results from the food analysis and the digestibility trial will be discussed initially. The results from the diet selection experiment will subsequently be discussed with reference to the results from the food analysis.

2.5.1 *Food analysis*

The results on the CP and urea analysis shown in Table 2.4, clearly demonstrate a change in the chemical composition of foods from the start, to the end, of the experiment. This change in the chemical composition was thought to be due to the separation of the food ingredients within the one tonne tote bags where the foods were stored. The colour of the foods at the surface changed though out the experiment. It was thought that the heavier food ingredients such as the Barley, that is darker in colour than oatfeed, moved towards the bottom of the bag. By the end of the experiment, the CP contents of the foods initially supplemented with 0, 7.5 and 15g urea/kg for both the L and H series were similar. Foods supplemented with 22.5g urea/kg did have a decrease

in both CP and urea content but still maintained a CP and urea content greater than those measured for all other foods. The results from the diet selection experiment could be expected to show diet preference for food $H_{22.5}$ when offered as a choice along with any other food from the H series as it is expected that there was adequate fME to support the additional nitrogen supplied by urea. The results could also be expected to show an avoidance for food $L_{22.5}$ when offered as a choice with any other food from the L series as it was predicted that there would not be enough fME in the L food to support the additional nitrogen supplied by urea.

The initial reason that the foods were present in the form of a meal was to prevent any changes in the chemical composition of the foods that may occur during the pelleting process (Van Soest, 1982). Pelleting foods can result in the condensation of sugar residues with amino acids followed by polymerisation to form a brown substance. This process, known as the Mailard reaction, renders protein indigestible. Heating the foods above 60^0C in the presence of steam, such as during the pelleting process, has been shown to increase the speed of this reaction. Therefore, as the experimental objective was to determine whether sheep selected for an optimal ratio between fME (which includes sugars) and eRDP (which includes amino acids), it was decided that the foods would not be pelleted. However, as described, this resulted in other problems such as ingredient separation. Therefore, during future experiments, all foods offered to sheep will be pelleted. To try and reduce the occurrence of the Mailard reaction, or other chemical reactions triggered off by heat and moisture, both heat and moisture were kept to the minimum possible during the pelleting process.

2.5.2 *Digestibility trial*

Oatfeed, of which L contained a greater proportion of than food H, has previously been demonstrated to have a lower DM and NDF digestibility than barley of which the food H contained a greater proportion (MAFF, 1990). In addition, increasing the quantity of food eaten by a ruminant animal generally causes a faster rate of passage of digesta. The

food is then exposed to the action of microbial fermentation for a shorter period and therefore there maybe a reduction in its digestibility (Bines *et al.*, 1988). Therefore due to the ingredients of the H and L food, and the different food intake between these two foods, it was expected, and observed, that the digestibility of food L would be lower than that of food H.

The digestibility of foods may be reduced by deficiencies of nutrients such as a deficiency in the ammonia-nitrogen in the rumen liquor, as this will restrict microbial growth and thus fibre digestibility (Kanjanapruthipong and Leng, 1998; Carro and Miller, 1999). The addition of urea was expected to increase the microbial activity and hence fermentation and digestibility (Egan and Doyle, 1985; Manyuchi *et al.*, 1994) when added to a nitrogen deficient, yet fME adequate food. However, this was not the case, especially for the H food where fME was plentiful. There are two possible reasons for this. Firstly, as can be seen from the results in Table 2.4, the concentration of urea measured in the food at the end of the experiment was generally lower than anticipated. The additional nitrogen, actually supplied by the urea, therefore may not have been sufficient to result in an increase in DM digestibility. Secondly, the CP content of the food was greater than expected. Therefore the foods may not have been deficient in RDP (AFRC, 1993), and the supplementation of urea would therefore not be of any benefit (Shain *et al.*, 1998).

2.5.3 *Diet selection experiment*

The hypothesis of this experiment was that ruminants select a diet to obtain an 'optimal' eRDP: fME ratio that would maximise microbial protein synthesis yet minimises costs such as those associated with the removal of excess RDP. This hypothesis was based upon the fact that in many instances the majority of the protein that is absorbed from the digestive tract of ruminants is of microbial origin (Nolan and Leng, 1972; Dijkstra et al., 1998). However, due to the changes in the chemical composition of the foods from the start of the experiment to the end, it was impossible to estimate the eRDP: fME ratio

selected by the sheep daily, as it was not know at what point, and to what degree at each point, the food ingredients separated and their chemical composition changed. Therefore the results from the diet selection experiment can only be viewed in terms of whether sheep appeared to select a diet in the general direction of that predicted.

2.5.3.1 *Sheep offered one food*

The H food was designed to be deficient in eRDP yet contain adequate fME to support additional eRDP supplied by urea. It was originally expected that the urea supplementation, hence an increase in eRDP, would result in an increase in food intake (Ørskov *et al.*, 1971; Egan and Doyle, 1985; Manyuchi *et al.*, 1994). However, as the CP content of foods H, $H_{7.5}$ and H_{15} were similar, food intake was subsequently not expected to differ between sheep offered these foods. Food $H_{22.5}$, however, contained a greater content of measured CP, and potentially more eRDP, than any other foods from the H series, yet no increase in food intake was observed. This may have been due to the CP and presumable the eRDP content of the H basal food, prior to urea supplementation, not being limiting as the CP measured at the start of the experiment was greater than initially predicted.

Food L was designed to be deficient in eRDP with respect to the fME content of the food (AFRC, 1993). However, as the fME content of food L was lower than that of food H, less eRDP was required to obtain the estimated 'optimal' eRDP: fME ratio of 10:1. However, as with the H food, the CP and presumably the eRDP content of the basal food, prior to urea supplementation at the start of the experiment, was greater than predicted. Therefore there is a strong possibility that the L food may not have been severely protein deficient.

The CP and urea content of food $L_{22.5}$ was greater than that of any of the other foods from the L series and could potentially have contained surplus RDP. Urea supplemented to concentrate foods that are calculated to contain adequate eRDP: fME rarely results in a significant change in food intake (Shain *et al.*, 1994). It is possible that the sheep in the

experiment reported here and the cattle in Shain *et al.*'s (1994) experiment coped with the additional RDP from urea, and hence the expected increase in rumen ammonia concentration, by changing their feeding behaviour (Loosli and Warner, 1958; Dulphy *et al.*, 1992).

A significant finding from the results on the food intake of foods differing in their fME content was that sheep offered foods from the L series consumed more than sheep from the H series. Growing lambs and both pregnant and barren ewes have been shown to increase their intake of a food as the energy content of the food decreases (Cooper *et al.*, 1994; 1995). This was considered to be due to the sheep attempting to compensated for the decrease in the energy content of the food and subsequently maintaining their daily ME intake (Montgomery and Baumgardt, 1965; Lamming *et al.*, 1966). The increase in food intake by sheep offered foods from the L series may have, therefore, been due to the sheep attempting to maintain their required daily energy intake.

2.5.3.2 *Choice fed sheep*

As previously mentioned, the CP contents of the foods initially supplemented with 0, 7.5 and 15g urea/kg for both the L and H series were similar by the end of the experiment. However, foods supplemented with 22.5g urea/kg still contained greater concentrations of CP and urea than that measured for all other foods. Therefore the results from the diet selection experiment may show diet preference for food $H_{22.5}$ when offered as a choice along with any other food from the H series as it is expected that there was adequate fME to support the additional nitrogen supplied by urea. The results may also show an avoidance for $L_{22.5}$ when offered as a choice along with any other food from the L series, as it was predicted that there would not be enough fME in the L food to support the additional nitrogen supplied by urea.

To some extent it could be suggested that sheep offered H vs. $H_{22.5}$ showed a preference for the urea supplemented food and this may have been due to the sheep attempting to select an estimated 'optimum' eRDP: fME ratio. The argument that sheep select a diet to

obtain the estimated 'optimum' eRDP: fME ratio was strengthened by the results from sheep offered foods from the L series. When sheep were offered $L_{22.5}$ along with any of the other foods from the L series sheep showed a preference for the food with the lower urea supplementation and this preference was significant in two or the three groups (Kyriazakis and Oldham, 1993; Tolkamp *et al.*, 1998b).

Overall, the basal food (H or L) to which the urea was supplemented, significantly affected the proportion of lower urea supplemented food selected. When sheep were offered a choice of foods differing in their eRDP content from the H series, they showed a tendency to select the urea-supplemented food. However, when sheep were offered a choice of foods differing in their eRDP content from the L series, they showed a tendency to select the food without urea.

The results from the diet selection experiment are in the same direction as that suggested by the hypothesis. That is, when the fME content of the food is limiting, sheep avoid urea-supplemented foods, hence minimise excess nitrogen intake (Villalba and Provenza, 1997; Tolkamp *et al.*, 1998b). When the fME content of the food is in abundance sheep show preferences for the urea-supplemented foods. However, these results are far from conclusive due to the complications in the actual chemical composition of the foods offered. Therefore further work is required to determine is sheep will select their diet on the basis of eRDP content rather than MP yield as proposed by Tolkamp *et al.* (1998b).

Chapter 3

Effects of adding urea to foods with different protein contents

What we anticipate seldom occurs; what we least expect generally happens
Benjamin Disraeli (1804-1881)

3.1 Abstract

Two experiments were conducted in which growing sheep were given choices between foods differing in their contents of urea. The crude protein (CP) contents of both of the foods of the pair were also varied. The hypothesis tested was that the diets selected would be those that met the effective rumen degradable protein (eRDP) requirements of the animals and would avoid excess consumption of eRDP. Three basal foods, differing in CP were formulated. D was designed to be deficient in CP with 80g CP and 58g eRDP/kg DM; H was calculated to be adequate with 159g CP and 114g eRDP/kg DM; P had excess with 210g CP and 148g eRDP/kg DM. Other foods were made by adding 12.5 or 25g urea/kg fresh matter to each of the three basal to make a further 6 foods. In both experiments Texel x Greyface female sheep were used. In Experiment 1, 34 sheep weighing 37.2 (s.d.1.85) kg were randomly allocated to 1 of 6 choices. Groups 1 to 3 were offered pairs in the D series (D vs. D+12.5g urea/kg (n=6), D vs. D+25g urea/kg (n=6) and D+12.5g urea/kg vs. D+25g urea/kg (n=5)). Groups 4 to 6 were offered the same pairs of foods but with H instead of D. In Experiment 2, 96 sheep weighing 29.8 (s.d. 3.37) kg were randomly allocated to 1 of 12 groups. Groups 1 to 6 (n=6) were allocated a single food (D, D+25g, H, H+25g, P or P+25g urea/kg) throughout the experiment. Groups 7 to 12 were given a choice (n=10) between two foods. One food of the pair was the basal D, H or P. The other food was the same basal food supplemented with either 12.5 or 25g urea/kg. On the single foods adding urea to D resulted in an increase in food intake and live weight gain suggesting that D was deficient in eRDP. Adding urea to either H or P had no beneficial effects on intake or live weight gain. This suggests that both contained sufficient eRDP in relation to energy. Across all choice treatments in both experiments there was a highly significant preference ($p<0.01$) for the food with the higher urea content. In Experiment 1 0.62 (s.e. 0.04) of the diet selected was as the food with the higher urea content. In Experiment 2 the figure was 0.64 (s.e. 0.03). The general preference for the food with the higher urea content was unaffected by the CP contents of the foods used. The results do not support the hypothesis that sheep will avoid excess eRDP when given a choice and suggest that eRDP may not be a relevant dimension in diet selection in the conditions of these experiments.

3.2 Introduction

Our ability to predict and control the performance of ruminants, and their impact on the grazing environment, is limited to some extent by our inability to predict intake and the selection of a diet that will be made from the foods that are available. To understand and predict diet selection it is important to know what the animals are selecting for and against when they choose a diet.

Many experiments have addressed the question of whether animals have the ability to select a diet to meet their protein requirements when offered a choice. Such an ability has been demonstrated convincingly in pigs (Bradford and Gous, 1991; Kyriazakis and Emmans, 1991), laboratory rodents (Leshner *et al.*, 1972; Musten *et al*, 1974) and poultry (Rose and Kyriazakis, 1991; Forbes and Shariatmadari, 1994), but the results from experiments on ruminants have been variable. Such variability may reflect the greater complexity of the ruminant digestive system in dealing with protein. Cropper (1987), Kyriazakis and Oldham, (1993) and Gorgulu *et al.*, (1996) found that in a controlled environment, growing sheep selected a diet to meet their metabolisable protein (MP) requirements from two foods that differed in their MP yields. The test of adequacy was the performance of similar sheep on a series of single foods. These results were thus consistent with those on non-ruminant animals. However, pregnant sheep (Cooper *et al.*, 1994) and lactating cows (Tolkamp *et al.*, 1998a; Lawson *et al.*, 2000) offered two foods identical in ME yield but differing in their MP yields, selected diets that resulted in intakes of MP that exceeded their calculated MP requirements (Agricultural and Food Research Council (AFRC), 1993).

Tolkamp *et al.*, (1998b) proposed an alternative hypothesis to account for these apparently conflicting findings. They proposed that ruminants select their diet on the basis of effective rumen degradable protein (eRDP) content rather than MP yield. Ruminants have evolved a symbiotic system of digestion that involves microbial fermentation of foods prior to exposure to the host's own digestive enzymes (Van

Soest, 1994). The amino acid supply of ruminants comes largely from microbial protein, provided that the diet has sufficient eRDP (Van Soest, 1994). The hypothesis that ruminants will select a diet that meets the requirement for eRDP, which is captured and utilised by the microbes to produce microbial protein, is not therefore an unreasonable one.

Tolkamp *et al.*, (1998b) tested whether changes in the eRDP content of the foods offered affected diet selection in lactating cows. They concluded that the diet selected was consistent with the hypothesis that ruminants try to obtain an adequate, but not excessive, eRDP supply from their diet. However, the foods offered as a choice in this experiment differed in characteristics other than their eRDP content, including MP yield and food ingredients. Factors other than eRDP may thus have influenced the diets selected.

Two experiments were carried out to test the hypothesis that both deficient and excessive eRDP levels will be avoided. Urea was used as the additional eRDP source in foods with different crude protein (CP) contents in order to have no appreciable effect on the ingredient composition of the foods used. By using urea there was no distinction made between the eRDP and RDP scale. When calculating eRDP from RDP, it is assumed that 0.2 of the quickly degraded N is not captured by ruminal micro-organisms (AFRC, 1993). While eRDP is the relevant scale for requirements RDP would be the relevant scale for excess. The choices given were between foods of different urea contents, but of the same energy densities. Intensive systems of animal production, for both growing and lactating ruminants, involve feeding high levels of such concentrate foods. Simple experiments such as these are a necessary first step in understanding and predicting the diet selection for CP in ruminants in more complex situations, such as those found in grazing and natural systems.

3.3 Materials and Methods

3.3.1 *General*

The methodology shared by the two experiments is described first with the specific designs of each experiment given later.

3.3.1.1 *Animals and Housing*

Texel x Greyface female sheep were used. They had previously been housed as groups and fed hay of medium quality *ad libitum.* They were kept in individual pens (2 x 1.5m) with slatted floors in a shed that was naturally ventilated. Throughout Experiment 1, artificial lighting was provided from 0600 to 1800h but, as the experiment was carried out during March to May 1999 at latitude 56°N, the duration of daylight had the potential to be longer than the period of artificial lighting. Natural light was the only source of lighting used throughout Experiment 2 that ran from August to October 1999. The sheep were given 14 days acclimatisation to allow them to become accustomed to their new environment and experimental procedures before the start of the experiments. They were offered a high quality (CP 171g/kg DM; metabolisable energy (ME) 11.4 MJ/kg DM) concentrate *ad libitum* as a pellet. All sheep had free and continuous access to water throughout.

3.3.1.2 *Experimental Foods*

Three basal foods, D (deficient), H (high) and P (plentiful), that differed mainly in their CP and calculated eRDP contents were formulated (Table 3.1). The foods were presented as pellets to help to prevent selection within a food and also to make the appearance of the three foods as similar as possible. Food D was designed to be deficient in eRDP with respect to its fermentable ME (fME) content, and in MP, and to be unable to support potential growth when offered alone on an *ad libitum* basis (AFRC, 1993). Food (H) was intended to satisfy the requirements for eRDP with respect to the fME content of the food and also to more than meet the MP requirements of sheep of 37kg live weight for potential growth. Food (P), was

Table 3.1. *Ingredients and determined chemical analyses of the three basal experimental foods. Foods D and H were used in Experiment 1 and all three foods were used in Experiment 2. The calculated yields of ME and protein components are also given*

	Foods		
	D	H	P
Ingredients (g/kg)			
Wheat	300	359	224
Oatfeed	160	196	210
Citrus Pulp	449	180	170
Soya Bean Meal (solvent extracted)	0	170	303
50% Fat Premix†	25	25	25
Molasses	50	50	50
Salt	2	5	5
Dicalcium Phosphate	7	4	1
Limestone Flour	1.5	9	10
Vitamin and Mineral Mix‡	2	2	2
Sodium Sulphate	3.5	0	0
Total	1000	1000	1000
Chemical Analysis (g/kg DM)			
Dry Matter (g/kg)	872	875	880
Crude Protein	80	159	210
Crude Fibre	119	102	108
Modified Acid Detergent Fibre	191	159	170
Neutral Detergent Fibre	282	268	279
Ash	65	68	73
Calcium	9.1	9.3	9.3
Phosphorus	3.4	4.0	3.8
Sodium	2.5	2.6	2.6
Sulphur	2.3	2.2	2.4
Estimated yields§			
Metabolisable Energy (ME; MJ/kg DM)‖	11.9	11.9	11.9
Fermentable ME (fME; MJ/kg DM)	10.7	10.7	10.7
Metabolisable Protein (MP; g/kg DM)	49.3	97.8	111.4
ERDP (g/kg DM)¶	57.6	114.1	147.8
MP:ME (g/MJ)	4.1	8.2	9.4

† Manufactured and supplied by Central Farmers Ltd, Methil, Fife, Scotland.

‡ Vitamin and Mineral mix used was Scotmin ewe/lamb (Scotmin Nutrition Ltd, Ayr, Scotland).

§ Values calculated using the metabolisable protein system (AFRC, 1993) assuming rumen outflow rate of $0.05h^{-1}$ and standard values for degradability coefficients (MAFF, 1990).

‖ ME, calculated from food tables (MAFF, 1990).

¶ eRDP, effective rumen degradable protein.

designed to have excessive RDP with respect to the fME content of the food. For maximal protein synthesis, rumen micro-organisms of growing sheep are estimated to require 1MJ of fME to capture and utilise 10g eRDP (AFRC, 1993). The estimated contents of fME and ME were kept as similar as possible for the three foods.

Food H had a ratio of N to S of 14:1, which was calculated to satisfy the microbial requirements for sulphur (Agricultural Research Council (ARC), 1980; Qi *et al.*, 1994). The content of S in food D was similar to that in food H so that there was a surplus of S that would be sufficient to allow the additional N in the form of urea to be used when it was added to food D (see below). Additional S was not added to the H or P foods when supplemented with urea as it was expected that the additional N would not be captured and utilised by the micro-organisms as the fME content was limiting.

Six additional foods were formulated by adding either 12.5 or 25g urea/kg fresh matter to all three basal foods. Urea was used as a source of eRDP because it allowed a large change in the eRDP content of the food with no appreciable effect on the food ingredients (Table 3.2). 25g urea/kg was selected as the upper level as this addition of urea to D increased its eRDP content to that of the unsupplemented H food. 12.5g urea/kg was the intermediate concentration between 0 and 25g.

When urea was added to D, the calculated MP yield increased because there was sufficient fME available to support the additional N supplied by urea (Table 3.2). When D was offered as a choice with D supplemented with urea, it would be impossible to conclude whether the sheep were selecting a diet with respect to the MP or the eRDP content of the foods. However, addition of urea to H and P was not expected to increase the MP yield. Therefore, if H or P were offered as a choice with the same basal food supplemented with urea, the sheep would be given a choice between two foods differing in eRDP, but not MP, content.

Table 3.2. *The calculated effective rumen degradable protein (eRDP) and calculated metabolisable protein (MP) contents and the eRDP: fermentable metabolisable energy (fME) ratios of the three basal experimental foods, without and with the addition of 12.5 or 25g urea/kg fresh matter, used in Experiments 1 and 2.*

Basal Food	Urea added (g/kg fresh matter)	Calculated eRDP content (g/kg DM)	Calculated MP content (g/kg DM)	eRDP:fME (g/MJ)
D	0	57.6	49.3	5.4
	12.5	91.0	70.6	8.5
	25	124.4	80.9	11.6
H	0	114.1	97.8	10.7
	12.5	147.4	97.8	13.8
	25	180.7	97.8	16.9
P	0	147.8	111.4	13.8
	12.5	180.9	111.4	16.9
	25	214.0	111.4	20.0

3.3.1.3 *Experimental Procedure*

Each experiment consisted of 4 periods. Sheep were allocated to a pair of foods as described below for each experiment. The sheep had the opportunity to experience separately the two foods allocated (Kyriazakis and Oldham, 1993). Each of the two foods was offered alone for 2 weeks (periods 1 and 2). This enabled the sheep to gain experience of the foods to be offered as a choice. The order in which the sheep received the 2 allocated foods was balanced within a treatment. By offering each food alone for a period of two weeks, estimates of the effects of each individual food on food intake and the live weight gain could also be made. During periods 3 and 4 all sheep were given free and continuous access to both of the foods allocated. Periods 3 and 4 were each 2 weeks long during Experiment 1 and 3 weeks long during Experiment 2. The position of the 2 foods within a pen was balanced within a treatment. At the start of period 4 the position of the 2 foods was switched to determine if food position had an effect on diet selection.

3.3.1.4 *Measurements*

Fresh food was weighed and offered at 0830 hours each day and refusals were weighed and discarded. The live weights of the sheep were measured just prior to feeding on the first day of each experiment and weekly thereafter.

3.3.2 *Experiment 1*

Sheep approximately one year of age (n=34) and weighing 37.2(S.D. 1.85) kg were used. The 6 foods used in this experiment were the basal foods D and H supplemented with either 0 or 12.5 or 25g urea/kg. The sheep were randomly allocated to one of the 6 groups. Each group was allocated to a pair of foods. Groups 1 to 3 were offered foods from the D series, where the pairs were D vs. D+12.5g urea/kg (n=6), D vs. D+25g urea/kg (n=6) and D+12.5g urea/kg vs. D+25g urea/kg (n=5). Groups 4 to 6 were offered the same pairs of foods but with the H food instead of the D food. Group sizes for sheep offered the choices for the H series were the same as those offered a choice of the D series.

3.3.3 *Experiment 2*

Sheep approximately four months of age (n=96) and weighing 29.8(S.D. 3.37) kg were used. The 9 foods used in this experiment were the basal foods D, H and P supplemented with 0 or 12.5 or 25g urea/kg. The sheep were randomly allocated to one of twelve groups. Groups 1 to 6 (n=6) were allocated a single food (D, D+25g, H, H+25g, P or P+25g urea/kg) throughout the experiment. Single fed sheep were used to measure the effects of the basal food with or without urea supplementation on food intake and weight gain. Groups 7 to 12 were given a choice (n=10) between two foods. One food was the basal food, either D, H or P, and the other food was the same basal food but supplemented with urea at either 12.5 or 25g urea/kg.

3.3.4 *Food Analysis*

Samples of all foods offered were taken every week and a composite sample was analysed for DM, CP, neutral detergent (plus amylase) fibre (NDF) as described by Ministry of Agriculture, Fisheries and Food ((MAFF) 1993), acid detergent fibre (ADF), sodium, calcium and phosphorus contents (Table 3.1).

3.3.5 *Statistical Analysis*

All data were analysed using Minitab for Windows, release 11.1 (Minitab, Inc., 1996) unless otherwise stated.

For experiment 1 the changes in intake and weight gain between periods 1 and 2 were calculated for each sheep to test for treatment effects (basal food and urea supplementation) on food intake and weight gain when foods were offered alone. These data were analysed using a General Linear Model (GLM). Basal food (D or H), urea supplementation (0 and 12.5g, 0 and 25g and 12.5 and 25g urea/kg) and

order (the order in which sheep within a group received each of the 2 foods separately) were used as factors with initial live weight as a covariate.

For Experiment 2 the daily food intake data of sheep offered a single food throughout the whole experiment were pooled over all 10 weeks. They were analysed as a 3x2 factorial design (3 basal foods D, H and P; x 2 levels of urea supplementation 0 and 25g) with initial live weight as a covariate. For each sheep a simple linear regression was used over the 10 weeks to calculate daily live weight gain. The rate of gain and food conversion efficiency (FCE; g of weight gained/kg food eaten) were analysed in the same way as daily food intake.

GENSTAT for Windows, version 5.2 (Lawes Agricultural Trust, 1993) was used to fit an antedependence model for repeated measures (Kenward, 1987) to the data collected from the first week of the choice period (period 3). This was done to determine whether the order that the foods were offered during periods 1 and 2 had an influence on subsequent diet selection. The antedependence model was considered as the most appropriate for the data analysed since it provided a means of making comparisons between the groups at each time point (day), taking into consideration differences at previous time points.

Correlation coefficients (r values) were calculated between the composition of the diet selected for each day of the first week after the position change (beginning of period 4) and the composition of the diet selected in the final week of period 3, i.e. immediately prior to the change. A value of r of 1 indicates that the sheep followed the food perfectly when the position was changed. A value of r of zero shows that there was no relationship and a value of r of -1 indicates that sheep perfectly followed the position of the bin and not the food when food position was changed.

The diet selection data were normally distributed and were not transformed for further analysis in the interest of clarity. The data for daily food intake and diet selection, expressed as the proportion of total food intake taken as the lower urea

food, were analysed as a factorial design. The data for diet selection, from the second week of both periods 3 and 4 for experiment 1 and the second and third weeks of both periods 3 and 4 for experiment 2, were pooled for the statistical analysis (see results). For daily food intake and live weight gain the data for the whole of periods 3 and 4 in both experiments were used. In Experiment 1 there were 2 basal foods (D and H) and 3 urea choices (0 vs. 12.5g, 0 vs. 25g and 12.5g vs. 25g urea/kg) for each. In Experiment 2 there were 3 basal foods (D, H and P) and 2 urea choices (0 vs. 12.5g and 0 vs. 25g urea/kg) for each. In both initial live weight was used as a covariate. To test whether the proportion of food selected differed from random a Students 't'-test with a null hypothesis of mean = 0.5 for each group was used.

For each sheep a simple linear regression was used over the choice period (periods 3 and 4) to calculate daily live weight gain which was analysed in the same way as daily food intake for the choice period.

3.4 Results

3.4.1 *Experiment 1*

3.4.1.1 *Training period*

Increasing the amount of urea supplementation to a food resulted in an increase in daily food intake (Table 3.3), which was significantly greater than that when urea supplementation was decreased from period 1 to period 2 ($p<0.05$). The increase in food intake was greatest when sheep had previously been offered a food supplemented with 12.5g urea/kg and subsequently received a food supplemented with 25g urea/kg. The basal food to which urea was added, i.e. D and H had no significant effect on the change in daily food intake.

There was no significant effect of basal food, level of urea supplementation or the order in which the foods were offered on the change in live weight gain between periods 1 and 2. However, the interaction between basal food and the order in which the foods were offered was significant ($p<0.05$). When the amount of urea added to the D food was increased from period 1 to period 2, live weight gain was greater than when urea supplementation was decreased. However the opposite was true when urea was added to H.

3.4.1.2 *Choice period*

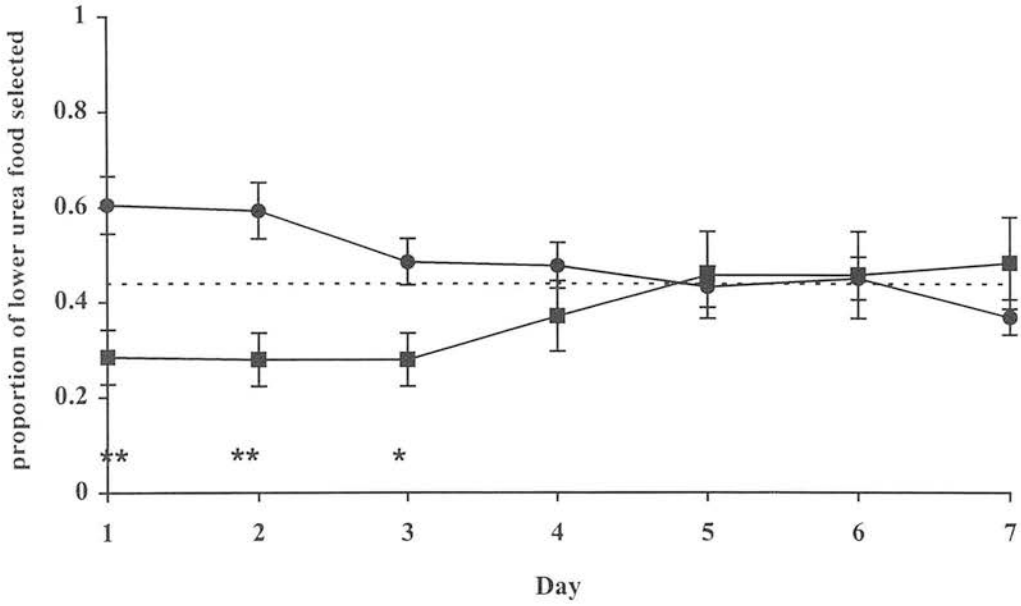
Figure 3.1a shows that sheep offered the food with the lower urea content during period 2 subsequently avoided the food with the lower urea content when offered a choice during the first three days of the choice period. The sheep offered the food with the higher urea content during period 2 chose a significantly higher proportion of the food with the lower urea content. As the food offered prior to the choice period influenced the diet selected over the first few days of the choice period, the data from the first week of the choice period were excluded from subsequent statistical analysis.

Table 3.3. *The change in daily food intake (g/d) and liveweight gain (g/d) between 2 periods when sheep consumed two different foods (Experiment 1). Both foods allocated were derived from the same basal food (D or H; D was deficient and H adequate in calculated eRDP) but differed in urea content (0 vs. 12.5, 0 vs. 25 or 12.5 vs. 25 g urea/kg fresh matter). The order in which sheep received the 2 foods was balanced within food pair.*

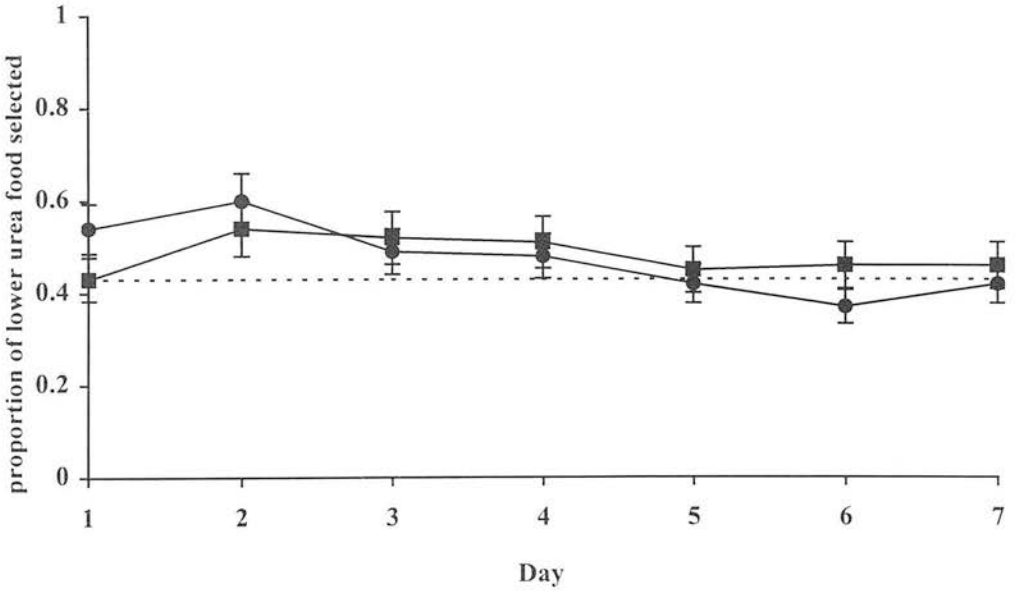
Food offered: Period 1	Period 2	Change in food intake between periods 1 and 2 (g/d)	Change in weight gain between period 1 and 2 (g/d)
D	D+12.5g urea/kg	200	197
D+12.5g urea/kg	D	52	-91
D	D+25g urea/kg	332	72
D+25g urea/kg	D	179	138
D+12.5g urea/kg	D+25g urea/kg	462	242
D+25g urea/kg	D+12.5g urea/kg	-25	131
H	H+12.5g urea/kg	99	-61
H+12.5g urea/kg	H	189	41
H	H+25g urea/kg	335	70
H+25g urea/kg	H	80	217
H+12.5g urea/kg	H+25g urea/kg	533	-50
H+25g urea/kg	H+12.5g urea/kg	61	119
s.e.d.		135	109
Basal		NS	NS
Pair		NS	NS
Order		*	NS
Basal*Pair		NS	NS
Basal*Order		NS	*
Pair*Order		*	NS
Basal*Pair*Order		NS	NS

Figure 3.1. *The effect of the food offered immediately prior to the choice period on the subsequent diet selected by sheep offered a pair of foods that differed in urea supplementation (0 vs. 12.5, 0 vs. 25 or 12.5 vs. 25g urea/kg) during Experiment 1(a) and Experiment 2(b). The food previously offered was either the higher ● or lower ■ urea supplemented food within a pair offered as a choice. The dotted line indicates the mean diet selected by both groups of sheep during days 5,6 and 7. (*, $p<0.05$; **, $p<0.01$).*

(a)



(b)



Across all six treatments there was a highly significant preference for the food with the higher urea content (Table 3.4). With one exception, each individual treatment showed this preference, although it was significantly different from random in only two cases. The exception was that where H was supplemented either with 12.5 or 25g urea/kg where there was a weak, and non-significant, preference for the food with the lower urea content.

There was a significant effect ($p<0.05$) of basal food on the daily rate of intake. Sheep offered foods from the D series consumed less than those offered food from the H series. Urea supplementation had no significant effect on daily rate of intake. There was no significant effect of treatment on live weight gain.

Figure 3.2 shows the effect of change of food position on the diet selected by sheep offered a choice of foods differing in urea content. The data are expressed as the proportion of the lower urea food selected during the week prior and the week following the position change. The r value for the week immediately preceding and the first day following the position change was 0.34 ($p<0.05$). By the 6th day after the position change the value of r value had increased to 0.66 ($p<0.01$).

3.4.2 *Experiment 2*

3.4.2.1 *Single fed sheep*

The daily food intake, live weight gain and FCE over the experimental period of 10 weeks for the single fed sheep are given in Table 3.5. Daily rate of intake was significantly affected by basal food ($p<0.001$) with sheep offered D having a significantly lower intake than that achieved by any other group. The addition of 25g urea/kg to D resulted in an increase in daily rate of intake but the amount consumed was still lower than that achieved by sheep offered the H or P foods. Urea supplementation to either H or P did not have a significant effect on the daily rate of intake.

Table 3.4. *The proportion of the lower urea food selected (kg/kg) from the second week of two choice periods (3 and 4), daily food intake (g/d) and liveweight gain (g/d) over all weeks from the choice periods by sheep offered a choice between two foods that differ in urea supplementation (0 vs. 12.5, 0 vs. 25 and 12.5 vs. 25g urea/kg fresh matter) during Experiment 1. The basal foods to which the urea was supplemented, D and H, were deficient and adequate in their calculated eRDP contents respectively with respect to the fME content of the food. Asterisks (*) next to the proportions of lower urea food selected denote that the diet selected differs significantly from random (0.5). The number in brackets is the standard error of the mean.*

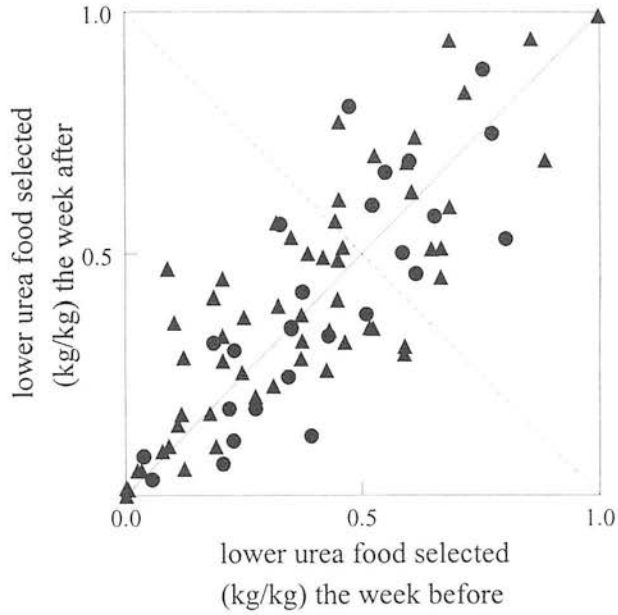
Food offered:	Proportion of the lower urea food selected (kg/kg)	Daily food intake (g/d)	Weight gain (g/d)
D vs. D+ 12.5g urea/kg	0.322*	1854	198
D vs. D+ 25g urea/kg	0.396	1777	197
D+ 12.5g vs. D+ 25g urea/kg	0.327	2044	241
H vs. H+ 12.5g urea/kg	0.298*	1979	245
H vs. H+ 25g urea/kg	0.270	2087	235
H+ 12.5g vs. H+ 25g urea/kg	0.642	2149	207
s.e.d.	0.108	146	35
Basal	NS	*	NS
Urea	NS	NS	NS
Basal*Urea	*	NS	NS
Overall mean	0.380 (0.0404)**		

Table 3.5. *The daily food intake (g/d), liveweight gain (g/d) and food conversion efficiency (FCE; g gained/kg food) over the 10 week experimental period of Experiment 2 by single fed sheep offered basal foods differing in calculated eRDP content (D was deficient, H adequate and P excessive with respect to the fME content of the foods) and urea supplementation (0 or 25g urea/kg fresh matter).*

Food offered:	Daily food intake (g/d)	Live weight gain (g/d)	FCE (g gained/kg food)
D	1366	149	103
D+25g urea/kg	1745	218	125
H	1940	278	149
H+25g urea/kg	1992	263	141
P	1984	325	165
P+25g urea/kg	2005	299	152
s.e.d.	99	21	7
Basal food	***	***	***
urea supplementation	*	NS	NS
Basal*urea	*	**	NS

The performance of the sheep offered D was lower than that of any other group. They had a lower daily weight gain and FCE. Supplementing the D food with 25g urea/kg improved performance but this was still inferior to that achieved by sheep offered H or P. Food P was significantly ($p<0.05$) superior to both the D and H food with respect to live weight gain and FCE. Urea supplementation to either H or P had no significant effect on performance.

Figure 3.2. *The diet selection chosen the week before against the diet selection chosen the week after the change in position of two foods offered as a choice for Experiment 1,● and Experiment 2,▲. The two foods offered as a choice differed in urea supplementation. The lines are those expected for sheep either following the food (——) or the position of the feed bins (----) when selecting their diet.*



3.4.2.2 *Choice-fed sheep*

3.4.2.2.1 *Training period.* The changes in the daily rate of intake between periods 1 and 2 were pooled across all groups that had an increase in urea supplementation or a decrease in urea supplementation from period 1 to period 2. The change in daily rate of intake was 248g/d for groups that had an increase, and 106g/d for groups that had

a decrease in the level of urea supplementation (s.e.d. 45 g/d; $p<0.01$). Increasing the amount of urea supplementation resulted in an increase in the daily rate of intake that was significantly greater than when urea supplementation was reduced from period 1 to period 2. As in Experiment 1, neither the basal food nor the amount of urea added had a significant effect on the change in daily rate of intake.

3.4.2.2.2 *Choice period.* The order that the sheep were offered their foods in periods 1 and 2 did not significantly influence the subsequent diet selection during the first few days of the choice period (Figure 3.1b). However, for consistency of analysis, the first week of the choice period was also excluded from further statistical analysis.

Across all six treatments there was a highly significant preference for the food with the higher urea content with only 0.364 (S.E. 0.028) of the selected diet as the food with the lower urea content of the pair (Table 3.6). This effect was present in all six cases although in only three was it formally significant. The preference for the food with the higher urea content was numerically stronger where the urea content was higher.

There was a significant effect ($p<0.001$) of basal food on the daily rate of food intake (Table 3.6). Sheep offered pairs of foods from the D series consumed at a lower rate than those offered pairs from the H and P series, with no difference between these two. Unlike the case in Experiment 1, the sheep offered the D series foods had a significantly lower weight gain than did the sheep offered either the H or P series foods. Urea supplementation had no significant effect on either food intake or live weight gain.

At the start of period 4 the position of the 2 foods within each pen were switched to determine if food position had an effect on diet selection (Figure 3.2.) The value of r for the week immediately preceding and first day following the position change was 0.40 ($p<0.05$). By the 4th day after the position change the value of r had increased to 0.55 ($p<0.01$).

Table 3.6. *The proportion of the non-urea food selected (kg/kg) from the second and third week of the two choice periods (3 and 4), daily food intake (g/d) and liveweight gain (g/d) from the whole of the choice period by sheep offered a choice between two foods that differ in urea supplementation (0 vs. 12.5 and 0 vs. 25g urea/kg fresh matter) during Experiment 2. The basal foods to which the urea was supplemented, D, H and P, were deficient, adequate and excessive in their calculated eRDP contents respectively with respect to the fME content of the food. Asterisks (*) next to the proportions of non-urea food selected denote that the diet selected differs significantly from random (0.5). Numbers in brackets are standard errors of the means.*

Foods Offered	Proportion of non-urea food selected (kg/kg)	Daily food intake (g/d)	Live weight gain (g/d)
D vs. D+ 12.5g urea/kg	0.373	1568	191
D vs. D+ 25g urea/kg	0.287**	1757	223
H vs. H+ 12.5g urea/kg	0.426	2061	277
H vs. H+ 25g urea/kg	0.333*	2069	266
P vs. P+ 12.5g urea/kg	0.422	1981	247
P vs. P+ 25g urea/kg	0.344*	1988	238
s.e.d.	0.098	119	24
Basal food	NS	***	**
Urea choice	NS	NS	NS
Basal food*Urea	NS	NS	NS
Overall mean	0.364 (0.0278)***		
Overall mean for 12.5g urea/kg	0.407 (0.0432)*		
Overall mean for 25g urea/kg	0.321 (0.0340)***		

3.5 Discussion

Animals are thought to have current output goals, such as growth rate and level of fatness, which they seek to achieve through their feeding behaviour (Kyriazakis, 1997). When given access to a choice of foods the animal will be expected to be able to choose the diet that allows these goals to be attained. Simple experiments are necessary as a first step to help to understand what the animal responds to when choosing a diet. The findings may then help in the understanding of diet selection in more complex situations, such as those found in natural grazing environments, and may even allow predictions to be made.

These experiments provided sheep with choices between foods differing in RDP. It was expected that when sheep were offered a choice between an eRDP deficient food (D) and the same food supplemented with urea, the supplemented food would be predominantly selected (Kyriazakis and Oldham, 1993). Sheep selecting the food supplemented with urea would be selecting a diet that met their eRDP requirements. When offered a choice between a food that satisfied the requirements of the sheep for eRDP (H or P), and the same food supplemented with urea, it was expected that the sheep would avoid the urea supplemented food (Tolkamp *et al.*, 1998b) in order to avoid excess RDP intake. The results obtained in the two experiments are discussed in turn against this background of expectations.

3.5.1 *Experiment 1*

During the first few days of the choice period the diets selected were greatly affected by the single food offered during the preceding period. Sheep generally preferred the food that had not been offered during the preceding period. However, although the effect was short lived, it is in the direction suggested by Provenza (1996) who proposed that there is a decrease in preference for the food just consumed as a result of sensory input unique to each food. Therefore on the first day that a different, but familiar, food is offered, sheep would be expected to select it. However, the observed

effect was longer lasting than would have been expected on the basis of sensory properties alone (Provenza *et al.*, 1996).

The sheep consistently, with one exception, preferred the food of the pair on offer that had the greater urea content (Table 3.4). These results were unexpected and contradictory to the view that one of the objectives of animals is to minimise excess in their selected diets (Kyriazakis, 1997; Kyriazakis *et al.*, 1999). The results could be accounted for by: (i) foods D and H were not deficient and adequate, respectively, in their actual eRDP contents as was intended; (ii) the nutritional history of the sheep influenced the diets selected; (iii) the true composition of the diets selected could not be adequately estimated because of the level of variation and the low level of replication.

Various authors have shown that supplementing a food deficient in eRDP with urea increases the rate of intake (Egan and Doyle, 1985; Manyuchi *et al.*, 1994) and live weight gain (Ørskov *et al.*, 1972; Kyriazakis and Oldham, 1993). Our results (Table 3.3) are in agreement. Live weight gain increased significantly to a greater extent when urea supplementation was increased from period 1 to period 2 compared to when urea supplementation was decreased. This is consistent with D being deficient in eRDP. On the other hand, when urea was added to H, a food calculated to have sufficient eRDP with respect to its fME content (AFRC, 1993), then the additional eRDP supplied by the urea was expected to be surplus to requirements. Excess ammonia needs energy for its removal, which in turn could result in a reduced live weight gain (Lobley *et al.*, 1995). This is consistent with the results obtained from sheep offered foods from the H series. Increasing urea supplementation from period 1 to period 2 resulted in a depression in live weight gain (-14 g/d) compared to sheep that had urea removed (126 g/d) from the H food offered from period 1 to period 2. This suggests that the H food contained adequate eRDP with respect to its fME content. Using changes in live weight gain were not ideal to determine whether the foods met our expectations but, due to the nature of the design, these data were all

that we had available. From these results it appeared that the foods used did meet the intended conditions needed for the treatments on diet selection.

A possible explanation for the diet selection results observed is the nutritional history of the sheep (Langlands, 1969; Newman *et al*, 1994). The Texel x Greyface sheep used weighed on average only 37kg at the start of the experiment, which is very low for their age of 12 months (Friggens *et al.*, 1997). They had been housed over winter and fed only medium quality hay *ad libitum*. This diet may have been deficient leading to their low live weight. How this might have influenced their diet selection is, however, unclear. It is of course possible that other specific factors could have been responsible for the results such as live weight, age and the time of the year.

With at least five sheep in each group we expected, on the basis of past results (Cooper *et al.*, 1995; Chapter 5), to establish satisfactory significant differences, where they existed. However, the standard errors of the proportion of a food in the diet selected were higher than those in other experiments using sheep of the same breed, kept in a similar environment, with the same size groups (Arsenos, 2000). Had the animals been unable to distinguish between the two foods of the pair then there could have been large variation between animals. However, this was not the case as the sheep followed the foods when their positions were switched. Experiment 2 was designed to overcome the shortcomings of the first experiment that could have led to the unexpected diet selection.

3.5.2 *Experiment 2*

3.5.2.1 *Single fed sheep*

Sheep did not compensate when offered the low protein food D by increasing their rate of food intake as seen in simple stomached animals (Kyriazakis *et al.*, 1990). As a result, live weight gain was significantly lower than that achieved on all of the other single foods. Foods deficient in eRDP can cause decreases in microbial activity, microbial growth (Kanjanapruthipong and Leng, 1998), rate of fermentation

and, as a consequence, a physical limitation may then reduce food intake (Newbold, 1987). The fact that the addition of urea, and hence eRDP, to the D food resulted in an increase in food intake and in live weight gain (Table 3.5), as in Experiment 1, shows that food D was deficient in eRDP.

Intake on both H and P was greater than on D (Table 3.5). This was expected as foods H and P were expected to yield more MP than food D as the latter was formulated to be deficient in MP (AFRC, 1993). Additions of urea to H had no effect on daily food intake or live weight gain (Shain *et al.*, 1998). There was no evidence of the reduction in growth rate that was indicated by the change over of foods from period 1 to 2 during Experiment 1. The results from the control groups in Experiment 2 were over a longer period of time and there were thus little chance of a carry over effect from a food previously consumed. Therefore it was considered that the results from Experiment 2 were a better indication of the effects of the single foods on intake and live weight gain. It is important that such effects are quantified for the single foods used in diet selection experiments (Emmans, 1991). The results suggest that the eRDP content of H was adequate but also indicate that there were no measurable costs to the animal associated with supplementing H with urea with respect to food intake and live weight gain.

As the fME content of foods H and P were similar, P contained excess eRDP. However, the addition of urea to P did not result in a decrease in the rate of food intake as had previously been shown in foods with limiting fME (Nomani, 1973; Dulphy *et al*, 1992). In other experiments sheep were able to cope with an excess of eRDP with no measurable effects on daily live weight gain (Milton *et al.*, 1997; Shain *et al.*, 1998). Ammonia concentration in the rumen and portal vein may act as a 'signal' that is used to determine food intake and diet selection (Chamberlain and Choung, 1995; Parker *et al.*, 1995). It is possible that the animal can cope with such problems by changing its feeding behaviour, at least to some extent. More frequent, and smaller, meals may allow the extent of the rise in ammonia concentration to be reduced (Dulphy *et al.*, 1992; Kahn, 1996). In these experiments suitable equipment

(Tolkamp *et al*, 1998a; Bornett *et al*, 2000) was not available to measure the daily pattern of intake.

3.5.2.2 *Choice-fed sheep*

The sheep given a choice of foods had the prior opportunity to experience separately the two foods to be given. The composition of the food given immediately prior to the choice did not affect the composition of the diets selected during the first few days of the choice period, as had been the case in Experiment 1. This may have been because the younger sheep used were able to learn more quickly where each of the foods was positioned, and to associate the physiological consequence of each food with its sensory and positional qualities, than could the older sheep of the previous experiment. This is consistent with the finding of Gower and Lamberty (1993) in mice.

The compositions of the diets selected were consistent between experiments (Tables 3.4 and 3.6). The sheep in both experiments showed a strong general preference for the food with the greater concentration of urea regardless of the food to which the urea was added. The observed changes when the position of the troughs were reversed at the end of period 3 showed that it was food composition and not food position that was affecting diet selection. This is in agreement with Cropper (1987), Kyriazakis *et al* (1990) and Hou (1991). In addition, the differences in the nutritional histories or age of the sheep in the two experiments did not affect diet selection.

The preference for the food with the higher urea content tended to be stronger when the supplemented food had 25g rather than 12.5g urea/kg, which was unexpected. It has been shown that, given the opportunity, ruminants will try to avoid an excess consumption of rapidly degradable protein (Villalba and Provenza, 1997; Tolkamp *et al.*, 1998b). In both experiments reported here sheep did not try to minimise any excess consumption of RDP when supplied as urea. In fact they chose the foods with the greater quantity of urea. The average ratio of eRDP:fME in the diets selected

increased from 15g/MJ for the choice H vs. H+25g urea/kg, to 18g/MJ for the choice P vs. P+25g urea/kg. These ratios are much higher than the 11g/MJ which is considered to be the requirement (AFRC, 1993). We therefore need to consider the possibility that there was some property of urea, other than its contribution to RDP, to which the sheep on the H and P series were responding.

Ruminants have evolved so that they are able to utilise forages and fibrous roughage (Van Soest, 1994). The foods used in the two experiments reported here were low in fibre, of high fermentability and were pelleted. On such foods a reduction in rumination (Campion and Leek, 1997) and a lower pH in the rumen would be expected (Cooper *et al*, 1995; Chapter 5). This could lead to a lower than optimum rumen pH (Owens *et al.*, 1998). It has been suggested that one of the objectives of ruminants when selecting a diet is to maintain the rumen environment within a certain physiological range (Parsons *et al.*, 1994; Cooper *et al.*, 1995; Kyriazakis, 1999). It is possible that the diets selected by the sheep were, at least in part, a means of reducing the potential disturbance of the rumen environment and had nothing to do with protein either as eRDP or as MP.

Haaland *et al.* (1982) found that the buffering capacity of the rumen fluid was increased when the crude protein content of the food was increased from 14 to 17%. Urea may also act as in a similar way through the mild buffering effects of ammonia, as suggested by Poos *et al.*, (1979). There is a possibility that the sheep in the experiments reported here, when offered a diet deficient in eRDP (the D series), selected the food supplemented with urea in order to minimise the deficiency in eRDP (Kyriazakis and Oldham, 1993). However, when the foods offered as a choice contained adequate eRDP (the H and P series), sheep may have selected for the foods supplemented with urea, because of their ability to buffer the rumen environment and maintain a higher pH (Phy and Provenza, 1998) on the high quality foods used. The buffering ability of the urea would help to reduce the disruption to the rumen environment, such as a fall in rumen pH, brought about by the low fibre and

roughage contents of the foods used. This hypothesis could not be tested further in this experiment.

The experiments reported here were designed as simple tests of the ability of growing sheep to select diets from foods differing in eRDP content. Even for the simple case chosen there appears to be no existing theoretical framework that would have predicted the results found here. In order to predict accurately the diets that ruminants will select we need to identify the dimensions that are relevant. It is possible that the urea was acting as a buffer. The questions then are: will ruminants, when offered the same choices of foods as in these experiments, change their diet selection when the foods are supplemented with a buffer? Will diet selection change if forage is also offered? In such cases urea may no longer be needed as a buffer and the preference for the urea supplemented food may disappear. Experiments are needed to test these possibilities.

Chapter 4

Sodium bicarbonate, but not the offering of hay, modifies the effect of urea on diet selection

There are as many opinions as there are experts.

Franklin D. Roosevelt (1882-1945)

4.1 Abstract

The hypotheses tested were that the expected preference of sheep for a food with adequate rumen degradable protein (RDP) supplemented with urea would be reduced both by the addition of a buffer (sodium bicarbonate (SB)) and by offering *ad libitum* access to hay. A control food (C), calculated to be adequate in its ratio of effective RDP to fermentable metabolisable energy (fME), was formulated. Other foods were made by adding 12.5 (U_1) or 25 (U_2) g urea/kg fresh matter (FM) to C and 20g SB/kg FM to C, U_1 and U_2. The acid buffering capacity (ABC) of each food was measured *in vitro*. The experiment consisted of two successive periods, each of 4 weeks. Ninety-eight female, Texel x Greyface sheep were randomly allocated to 14 groups each with n=7. Groups 1 to 6 were offered one of: C, U_2, C+SB, U_2+SB, C with hay or U_2 with hay throughout the experiment. Groups 7 to 10 were offered the choices of C vs. U_1 or C vs. U_2, either with or without hay in a change-over design; animals that received hay during period 1 (groups 8 and 10) did not during period 2 and vice versa (groups 7 and 9). Groups 11 to 14 (n=7) were offered the choices of C vs. U_1 or C vs. U_2, either with or without SB supplemented to both foods, in a change-over design. Adding either urea, or SB, or both to C had no beneficial effects on intake or live weight gain when offered alone. Both supplements significantly ($P<0.001$) increased the ABC of food C. Throughout the experiment hay consumption was very low (overall mean: 23 (s.e. 2.5)g hay/sheep day). Offering hay caused no change in the preference for the urea-supplemented foods. Sheep offered the choices C vs. U_1 or C vs. U_2, with neither hay nor SB, selected 0.466 (s.e. 0.036) and 0.593 (s.e. 0.025) kg/kg total food intake (TFI) of U_1 and U_2 respectively. The proportions of the urea-supplemented foods were significantly reduced ($P<0.01$) by SB supplementation: 0.348 (s.e. 0.045) and 0.406 (s.e. 0.059) kg/kg TFI of U_1 and U_2 respectively. The effect of SB addition on the diet selection of sheep could be due to its buffering properties. When SB is added to both foods the need for urea to be used as a buffer is reduced with a consequent decrease in the proportion selected as the urea-supplemented food. Effects of diet on buffering may override other diet selection objectives, such as the avoidance of an excess intake of RDP.

4.2 Introduction

Animals can be seen as having current output goals, such as growth rate, which they seek to achieve through their feeding behaviour (Kyriazakis, 1997). When given access to a choice of foods the animal is expected to be able to choose the diet that allows these goals to be attained. It has been shown that non-ruminants can select a diet that meets their protein requirements (Leshner *et al.*, 1972; Bradford and Gous, 1991; Kyriazakis and Emmans, 1991; Forbes and Shariatmadari, 1994) when given an appropriate choice. Animals may also, in at least some cases, avoid an excess consumption of protein when they are given a choice (Musten *et al.*, 1974; Kyriazakis *et al.*, 1990). The expectation then is that animals will select a diet that both meets their protein requirements and avoids excess when offered an appropriate choice (Emmans, 1991). However, the results from experiments on ruminants have not been consistent with this view and this inconsistency may reflect the greater complexity of the ruminant digestive system in dealing with protein. It can be presumed that ruminants also use some rules for selecting their diet from the foods available. Under natural conditions there will often be many foods that can be eaten and it is difficult both to measure the diets selected and to use the observations to test ideas about the rules being used (Kyriazakis *et al.*, 1999). Two-way choices under controlled conditions offer a starting point in the understanding of diet selection in ruminants, as in other animals.

Even where only two foods are used that are intended to differ in only one or two dimensions, the problem of accounting for the observed choices is not an easy one to solve (e.g. Tolkamp *et al.*, 1998a). The conflicting evidence for the effect of food protein content on choice is an example. Tolkamp *et al.* (1998b) proposed an alternative hypothesis to the view that animals select a diet in order to meet their protein requirements, to try to account for the conflicting findings. They proposed that ruminants select their diet on the basis of effective rumen degradable protein (eRDP) content rather than on metabolisable protein (MP) yield. As the protein supply of ruminants comes largely from microbial protein (Van Soest, 1994), the

idea that they may select a diet that meets their requirement for eRDP is not an unreasonable one. However, ammonia resulting from protein degradation in the rumen in excess of the capacity of the microbes to capture and utilise it would be associated with costs (Lobley *et al.*, 1995; Parker *et al.*, 1995) and could even be detrimental (Chalupa, 1968; Visek, 1968). Therefore it would be advantageous also for the ruminant to avoid an excess consumption of RDP.

Two previous experiments (Chapter 3) tested the hypothesis that growing sheep would select a diet that met their requirements for eRDP, but would avoid an excess consumption of RDP. Urea was used as the eRDP source in foods with different crude protein (CP) contents. The choices offered were always between foods with different urea contents. Unexpectedly, sheep showed a consistent preference for the food with the greater urea concentration, regardless of the food to which it was added. The sheep therefore did not try to minimise any excess consumption of RDP when supplied as urea, but positively selected for excess. This is inconsistent with the findings from other experiments (Kyriazakis and Oldham, 1993; Tolkamp *et al.*, 1998b).

One possible explanation for the unexpected findings is that the sheep in the experiments of Chapter 3 is that the sheep were offered concentrate foods only with no long forage. This would result in a reduction in rumination (Welch, 1967; Campion and Leek, 1996; 1997), which in turn would be expected to reduce the amount of saliva entering the rumen (Bailey, 1961) and hence its pH (Dougherty *et al.*, 1975; Russell *et al.*, 1979). It is thus possible that the sheep were selecting diets high in urea in order to increase rumen pH. It has been shown that urea can increase rumen pH (Kyriazakis and Oldham, 1997). Ruminants may try *both* to maintain rumen pH above a certain value *and* to avoid an excess RDP intake. Where these two objectives conflict it is not clear which will take priority.

The objective of the experiment was to test two hypotheses. H1: the proportion of a urea supplemented food in the selected diet would be reduced by offering hay *ad*

libitum along with the choice of concentrate foods. H2: the proportion of a urea-supplemented food in the selected diet would be reduced by supplementing with sodium bicarbonate ($NaHCO_3$) both foods offered as a choice. In both cases urea may no longer be needed as a buffer to the same extent and preference for the urea-supplemented food would then be expected to be reduced or even disappear.

4.3 Material and Methods

4.3.1 *Animals and housing*

Ninety-eight Texel x Greyface female sheep, approximately one year of age and weighing 35.5 (s.d. 4.3) kg, were used. They were housed in individual pens (2 x 1.5m) in a slatted floor shed that was naturally ventilated. Natural light was the only source of lighting used throughout the experiment that ran from March to May 2000 at latitude 56°N. The sheep were given 14 days acclimatisation to allow them to become accustomed to the new environment and procedures before the start of the experiment. During this period they were offered *ad libitum*, a high quality pelleted food with 171g CP/kg dry matter (DM) and 11.4 MJ metabolisable energy (ME)/kg DM with no access to long forage. All sheep had free and continuous access to water throughout.

4.3.2 *Experimental foods*

The control food C contained 349g wheat, 196g oatfeed, 180g citrus pulp, 170g high protein soya bean meal (solvent extracted decorticated soya bean), 25g 50% fat premix (manufactured and supplied by Central Farmers Ltd, Methil, Fife, Scotland), 50g molasses, 15g salt, 4g dicalcium phosphate, 9g limestone flour and 2g vitamin and mineral mix (Scotmin ewe/lamb, Scotmin Nutrition Ltd., Ayr, Scotland) per kg. It had 880g DM/kg and 176g CP/kg DM. It was calculated to satisfy the requirements for eRDP (114 g eRDP/kg DM) with respect to the calculated fME (10.7 MJ ME/kg DM) content of the food (eRDP: fME: 10.7g/MJ), and also to, at least, meet the MP requirement of sheep of 36 kg live weight for potential growth (Agricultural and Food Research Council (AFRC), 1993). The MP yield was estimated to be 98g MP/kg DM with an MP:ME ratio of 8.2g/MJ. The food was presented as pellets to help to avoid selection within the food. Food C had a ratio of N to S of 14:1, which was calculated to satisfy both microbial and sheep

requirements for sulphur (Agricultural Research Council (ARC), 1980; Qi *et al.*, 1994). Additional S was not added to C when supplemented with urea (see below).

Two additional foods were formulated by adding either 12.5 or 25g urea/kg fresh matter (U_1 and U_2 respectively) to C. Urea was used as a source of RDP because it allows a large change in the RDP content of the food with no appreciable effect on the ingredient composition. For microbial protein synthesis in growing sheep micro-organisms resident in the rumen are estimated to require 1MJ of fME to capture and utilise 10g eRDP (AFRC, 1993). Therefore it was expected that the additional N supplied by the urea would not be captured and utilised by the micro-organisms as the fME content would then be limiting. While eRDP is the relevant scale for requirements, RDP is the relevant scale for excess. In a previous experiment (Chapter 3) it was found that 25g urea/kg added to a food that was similar to C used in this experiment, did not result in signs of ammonia toxicity when it was fed alone. However, when offered along with the same food unsupplemented as a choice, there was a preference for the urea-supplemented food. For these reasons, and for consistency with the previous experiment, 25g urea/kg was used as the upper concentration with 12.5g urea/kg as the intermediate level.

Three further foods were formulated by adding 20g $NaHCO_3$/kg fresh matter to C (C+SB) and the two urea supplemented foods (U_1+SB and U_2+SB respectively). Sodium bicarbonate was selected for this experiment because many studies have previously shown that $NaHCO_3$ can increase rumen pH when added to foods of low fibre and high energy contents (Ha *et al.*, 1983; Hart and Polan, 1984; Denholm and Ling, 1987). The concentration of $NaHCO_3$ used in this experiment has previously been shown to increase food intake and rumen pH when added to a readily fermentable food (Ha *et al.*, 1983; Chapter 5). Much higher concentrations of $NaHCO_3$ could increase rumen osmolality that could result in a decrease in food intake as found by Carter and Grovum (1990) and Cooper *et al.* (1996). All of the six concentrate foods used in the experiment were adjusted to a common sodium content by adding sodium chloride to the foods not supplemented with $NaHCO_3$.

A medium quality hay (887g DM/kg; CP 77.6g/kg DM; crude fibre (MAFF, 1993) 363g/kg DM; ash 76.6g/kg DM) was used (see below). The hay was chopped using an industrial chopper (Agri-chop, Lauder, Scotland) to give a maximum chop length of about 12cm. The hay was chopped to minimise spillage when the sheep consumed it and to make weighing the hay easier.

4.3.3 *Food analysis*

Samples of all foods offered were taken every week and a composite sample was analysed for DM, CP, neutral detergent (plus amylase) fibre (NDF), acid detergent fibre (ADF) and sodium contents as described by MAFF (1993). The chemical analyses of the six foods, and of the hay used, are in Table 4.1.

The pH and *in vitro* acid buffering capacity of foods C, U_2, C+SB and U_2+SB were measured with and without urease at 37°C (as recommended by BDH Laboratory supplies, Poole, the manufacturers of urease-active meal, product 39045 25) using the procedure of Jasaitis *et al.* (1987). Urease was used to liberate the ammonia from the urea (Crawford *et al.*, 1983). The acid buffering capacities of water alone, water with urea, water with urease and water with both urea and urease were also measured as controls. Acid buffering capacity was calculated by dividing titratable acidity (total volume of acid added to each sample multiplied by its normality) by the total change in concentration of hydrogen ions ($[H^+]$ = 10 x (exp(-pH))). Thus, acid buffering capacity expresses the amount of acid required to produce a unit change in hydrogen ion concentration of each food sample.

4.3.4 *Experimental procedure*

The sheep were randomly allocated to one of 14 treatment groups (Table 4.2). Groups 1 to 6 (n=7) were allocated a single food throughout the experiment (8

Table 4.1. *Chemical analyses of the basal food (C) and C supplemented with 12.5 or 25g urea/kg (U_1 and U_2 respectively), these three foods supplemented with 20g $NaHCO_3$/kg (C+SB, U_1+SB and U_2+SB respectively) and the hay used in this Experiment. The calculated yield of effective rumen degradable protein (eRDP) is also given.*

	Foods						
	C	U_1	U_2	C+SB	U_1+SB	U_2+SB	Hay
Chemical Analysis (g/kg DM)							
Dry Matter (g/kg)	880	882	883	885	871	877	888
Crude Protein	176	211	232	167	208	242	77.6
Modified Acid Detergent Fibre	133	123	136	125	122	128	400
Neutral Detergent Fibre	198	194	218	198	190	206	663
Sodium	7.9	7.4	8.0	8.1	7.2	7.5	-
Estimated yield†							
ERDP (g/kg DM)	114	143	174	113	142	173	-

† Values calculated using the metabolisable protein system (AFRC, 1993) assuming rumen outflow rate of $0.05h^{-1}$ and standard values for degradability coefficients (MAFF, 1990).

Table 4.2. *The experimental treatments used; animals in groups 1 to 6 were offered a single food (n=7). Groups 7-14 (n=7) were given a choice between two foods. The experimental period of 8 weeks was divided into two, four-week periods.*

Group	Period 1 (weeks 1 to 4)	Period 2 (weeks 5 to 8)
1	C †	C
2	U_2 §	U_2
3	C + SB ‖	C + SB
4	U_2+SB	U_2+SB
5	C with hay	C with hay
6	U_2 with hay	U_2 with hay
7	C vs. U_1 ‡	(C vs. U_1) with hay
8	(C vs. U_1) with hay	C vs. U_1
9	C vs. U_2	(C vs. U_2) with hay
10	(C vs. U_2) with hay	C vs. U_2
11	C vs. U_1	C+SB vs. U_1+SB
12	C+SB vs. U_1+SB	C vs. U_1
13	C vs. U_2	C+SB vs. U_2+SB
14	C+SB vs. U_2+SB	C vs. U_2

† C, control food
‡ U1, control food supplemented with 12.5g urea/kg.
§ U2, control food supplemented with 25g urea/kg.
‖ +SB, the food supplemented with 20g $NaHCO_3$/kg.

weeks). They were offered one of: C or U_2, one of these two foods supplemented with $NaHCO_3$ (C+SB or U_2+SB) or one of these two foods with *ad libitum* access to hay (C with hay or U_2 with hay). Single fed sheep were used to measure the effects of each food on food intake and live weight gain. To minimise the number of groups, and therefore maximise group size, food U_1 was not offered singly.

Groups 7 to 10 (n=7) were offered a choice of foods (Table 4.2). The choices were between two foods, one with and the other without urea added, in the presence, or not, of hay. All choices were introduced abruptly. The crossover design used (see below) meant that a training period was not used. The intake data from the first week after a change were not used. Animals that received hay during period 1 (weeks 1 to 4) as a third choice did not do so during period 2 (weeks 5 to 8) and vice versa.

Groups 11 to 14 (n=7) were also offered a choice of foods (Table 4.2). The choices were between two foods, one with and the other without urea added, with or without $NaHCO_3$ added to both foods. Animals that received $NaHCO_3$ during period 1 (weeks 1 to 4) did not do so during period 2 (weeks 5 to 8) and vice versa. The positions of the two foods were randomised within treatment and their position was not changed throughout the experiment. When three foods were offered (i.e. two concentrate foods and hay), the food trough containing hay was positioned between the two troughs containing the concentrates.

4.3.5 *Measurements*

Food refusals were removed, weighed and discarded, and fresh food offered at 0830 hours each day. The live weights of the sheep were measured on the first day of the experiment and weekly thereafter.

4.3.6 *Statistical analysis*

All data were analysed using Genstat for Windows (release 3.2, Lawes Agricultural Trust, 1993) unless otherwise stated. Food pH and acid buffering capacity data were analysed as a 4x2 factorial design using ANOVA. Effects of, and interactions between food (C, U_2, C+SB or U_2+SB) and urease (with and without) were analysed with food and urease addition as factors.

4.3.6.1 *Single fed sheep*

The daily food intake data (not including hay) of sheep offered a single food throughout the whole experiment were pooled for the entire 8 weeks. These data were analysed as two, 2x2 factorial designs using ANOVA. For the first subset (groups 1 to 4), effects of, and interactions between, urea (0 or 25g urea/kg) and hay (0 or *ad libitum* access) were analysed with urea and hay as factors. For the second subset (groups 1, 2, 5 and 6), effects of, and interactions between, urea (0 or 25g urea/kg) and $NaHCO_3$ (0 or 20g $NaHCO_3$/kg) were analysed with urea and $NaHCO_3$

as factors. For each sheep the linear regression coefficient for weight on time, over the 8 weeks was used to estimate live weight gain. The rate of gain and food conversion efficiency (FCE; g of weight gained/kg food eaten) were analysed in the same way as daily food intake. Initial live weight was used as a covariate in all analyses of data from single fed sheep.

4.3.6.2 *Choice fed sheep*

To test for the presence of an effect of level of urea supplementation on diet selection, expressed as the proportion of total food taken as the urea supplemented food, the data from all sheep, on the treatments with neither $NaHCO_3$ nor hay, were used. These were groups 7, 9, 11 and 13 during Period 1, and groups 8, 10, 12 and 14 during Period 2 (Table 4.2). The effect was estimated with 28 sheep on each of the U_2 and U_1 choices. Effects of group and period, and interactions, were allowed for in the analysis. The diet selection data were normally distributed and were not transformed for further analysis. The data for the proportion of urea supplemented food selected from weeks 2 to 4 of each period were used for the statistical analysis. Diet selection data from the first week of each period were excluded as it was expected that this time period represented the time that sheep would be learning about the foods (Tolkamp and Kyriazakis, 1997; Chapter 3). These conditions were also applied to subsequent analyses of diet selection (below).

Using ANOVA, data from choice fed sheep for daily food intake (not including hay) and diet selections were analysed as two change over designs. In all analyses of data from choice-fed sheep, period was nested within sheep as a block factor to take into account the fact that the same sheep were used in both periods. The data from groups 7 to 10 were analysed for the effects of, and interactions between, period, urea choice (0 vs. 12.5 or 0 vs. 25g urea/kg) and hay (none or *ad libitum* access) with these three factors as treatments. The main null hypothesis was that hay would not modify the effect of urea on diet selection. The data from groups 11 to 14 were analysed for the effects of, and interactions between, period, urea choice (0 vs. 12.5 or 0 vs. 25g urea/kg) and $NaHCO_3$ (none or 20g $NaHCO_3$/kg) with these three factors as

treatments. The main null hypothesis was that $NaHCO_3$ would not modify the effect of urea on diet selection. To test whether the proportion of food selected differed from random (0.5) for a given group (n = 7), a Students 't'-test with a null hypothesis of mean = 0.5 was used (Minitab for Windows release 11.1, Minitab Inc., 1996). With only 7 sheep per group the test was not very powerful and we placed more reliance on the effects of treatment as described above.

For each sheep the linear regression coefficients for weight on time, over each four week choice period (periods 1 and 2), were used to estimate live weight gain. The rate of gain and food conversion efficiency (FCE; g of weight gained/kg food eaten) were analysed in the same way as daily food intake. Initial live weight was used as a covariate in all analyses of data from choice fed sheep.

4.4 Results

4.4.1 *Food pH and acid buffering capacity*

The buffering capacity of the water was very low at 0.117 (s.e. 0.0077) equivalents (eq) of acid required to produce a unit change in hydrogen ion concentration of the sample (eq/[H^+]). The buffering capacities of each food, with and without urease, are given in Table 4.3. The buffering capacity of food C was significantly lower than that of any other food. Sodium bicarbonate significantly increased the buffering capacity of the foods but not to the same extent as did urea in the presence of urease. The buffering effects of urea and $NaHCO_3$ were additive.

Table 4.3. *The* in vitro *acid buffering capacity of the control food (C), C supplemented with 25g urea/kg (U_2) and these two foods supplemented with 20g sodium bicarbonate/kg (C+SB and U_2+SB), with or without urease.*

Food	Acid buffering capacity† (eq)	
	Without urease	With urease
C	1.28	1.02
U2	2.07	6.59
C+SB	2.34	2.35
U2+SB	2.62	7.16
s.e.d	0.101	
Food	***	
Urease	***	
Food*urease	***	

†, Equivalents (eq) of HCl required to lower one unit of [H+] of 500mg dry matter suspended in 50ml distilled deionized water to 10^{-4} (pH 4), divided by total in [H+] change (initial concentration to 10^{-4} (pH 4)).

4.4.2 *Single fed sheep*

The daily food intake, live weight gain and FCE over the whole experimental period of 8 weeks for the single fed sheep are given in Table 4.4. Daily food intake was not

significantly affected by either urea supplementation or by addition of $NaHCO_3$, and there was no significant interaction between these two factors. Daily food intake was significantly ($P<0.001$) increased by offering hay *ad libitum*, even though only very small amounts of hay were consumed (Table 4.4). There were no significant interactions between urea and hay on food intake. There was no significant effect of single food on live weight gain or FCE.

4.4.3 *Choice-fed sheep*

4.4.3.1 *Effect of level of supplementation with urea*

Overall, with neither hay nor $NaHCO_3$ present, the preference for the food with the urea supplement was significantly ($P<0.01$) greater where the urea content was higher. Sheep selected (kg/kg total food intake) 0.466 of U_1 on C vs. U_1, and 0.593 of U_2 on U_2 vs. C, (s.e.d. 0.044; $P<0.001$). There was no effect of period or group on the proportion of urea-supplemented food selected, nor were there any interactions. While the sheep on U_2 vs. C selected significantly ($P<0.001$) more than 0.5 of the food with urea those on U_1 vs. C did not. The level of urea supplementation had no significant effects on food intake, live weight gain or FCE.

4.4.3.2 *Effect of offering hay on diet selection and performance*

Offering hay *ad libitum* to groups offered a choice between two concentrate foods did not result in a decrease in preference for the food supplemented with urea, and thus did not significantly affect diet selection. In none of the four groups did the diet selected differ significantly from random (Table 4.5).

Daily food intake was not affected by offering hay (Table 4.5). For the groups offered hay in periods 1 or 2, there was a significant increase ($P<0.001$) in food intake from period 1 to period 2. Sheep consumed on average 2040 and 2371 (s.e.d. 81) g/d during period 1 and 2 respectively. The consumption of hay remained low throughout the experiment at about 15g/d. Offering hay *ad libitum*, had no effect on daily live weight gain nor on FCE. However, live weight gain and FCE were both

Table 4.4. *The daily food intake (g/d), live weight gain (g/d) and food conversion efficiency (FCE; g gained/kg food) over the 8 week experimental period by single fed sheep offered one of: the control food (C) or C supplemented with 25g urea/kg (U_2), one of these two foods supplemented with $NaHCO_3$ (C+SB or U_2+SB) or one of these two foods with* ad libitum *access to hay (C with hay or U_2 with hay). Numbers in brackets indicate the amount of food consumed as hay (g/d).*

Group	Food offered:	Daily food intake (g/d)	Live weight gain (g/d)	FCE (g gained/kg food)
1	C	2056	307	159
2	U_2	2136	324	174
3	C+SB	2175	344	171
4	U_2+SB	2213	317	149
5	C with hay	2385 (33)	343	150
6	U_2 with hay	2345 (22)	360	150
	Comparisons between urea and SB supplementation (groups 1-4)			
	s.e.d.	127	33	10
	Urea	NS	NS	NS
	SB	NS	NS	NS
	Urea*SB	NS	NS	NS
	Comparisons between urea and offering hay *ad libitum* (groups 1, 2,5 and 6)			
	s.e.d	110	36	11
	Urea	NS	NS	NS
	hay	**	NS	NS
	Urea*hay	NS	NS	NS

Table 4.5. *The proportion of the urea supplemented food selected (kg/kg total food intake, TFI), excluding the first week of each of the two choice periods (P1 and P2), daily food intake, live weight gain and food conversion efficiency (FCE) over all weeks of the two choice periods by sheep offered a choice between two foods that differ in urea supplementation (0(C) vs. 12.5(U_1) or 0 vs. 25(U_2) g urea/kg) with or without* ad libitum *access to hay. The numbers in brackets indicate the amount of food consumed as hay (g/d).*

Choice offered		Proportion of urea supplemented food selected (kg/kg TFI)		Food intake (g/d)		Live weight gain (g/d)		FCE (g gained/kg food)	
P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
Cvs.U_1	Cvs.U_1 with hay	0.488	0.483	1997	2369 (16)	410	307	205	129
Cvs.U_1 with hay	Cvs.U_1	0.494	0.506	1969 (18)	2108	363	331	185	154
Cvs.U_2	Cvs.U_2 with hay	0.555	0.582	2121	2408 (13)	319	257	151	107
Cvs.U_2 with hay	Cvs.U_2	0.576	0.567	2075 (15)	2386	362	283	172	119
	s.e.d	0.090		146		68		31	
	Significance of† :								
	Period	NS		***		*		**	
	Urea	NS		NS		NS		NS	
	Hay	NS		NS		NS		NS	

† None of the interactions is significant.

significantly lower ($P<0.05$ and $P<0.01$ respectively) in period 2 (312 g/d and 133 g gained/kg food respectively) than in period 1 (364 (s.e.d.30) g/d and 178 (s.e.d. 12) g gained/kg food respectively).

4.4.3.3 *Effect $NaHCO_3$ supplementation on diet selection and performance*

The proportion of the urea-supplemented food in the diet selected was reduced in all four cases (Table 4.6) by supplementing both foods with $NaHCO_3$. None of the interactions between period, level of urea and $NaHCO_3$ was significant. Overall the supplementation with $NaHCO_3$ decreased the proportion of the urea-supplemented food in the diet selected from 0.525 to 0.377 ($P<0.01$).

Daily food intake was not affected by the addition of $NaHCO_3$ (Table 4.6). For the groups offered $NaHCO_3$ in period 1 or period 2, there was a significant increase ($P<0.001$) in food intake from period 1, 2122 g/d, to period 2, 2425 g/d (s.e.d. 38). Supplementation with $NaHCO_3$ had no effect on either daily live weight gain or FCE. However, live weight gain and FCE were both significantly lower ($P<0.05$ and $P<0.001$ respectively) in period 2 (302 g/d and 126 g gained/kg food respectively) than in period 1 (375 (s.e.d.32) g/d and 177 (s.e.d.13) g gained/kg food respectively).

Table 4.6. *The proportion of urea supplemented food selected (kg/kg total food intake, TFI), excluding the first week of each of the two choice periods (P1 and P2), daily food intake, live weight gain and food conversion efficiency (FCE) over all weeks of the two choice periods by sheep offered a choice between two foods that differ in urea supplementation (0(C) vs. 12.5(U_1) or 0 vs. 25(U_2)g urea/kg) with or without sodium bicarbonate (+SB) supplementation (20g SB/kg). Asterisks (*) next to the proportion on non-urea food selected denote that the diet selected differed significantly from random (0.5).*

Choice offered		Proportion of urea supplemented food selected (kg/kg TFI)		Food intake (g/d)		Live weight gain (g/d)		FCE (g gained/kg food)	
P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
C vs. U_1	C+SB vs. U_1+SB	0.480	0.405	2077	2425	382	295	185	123
C+SB vs. U_1+SB	C vs. U_1	0.291*	0.388	2094	2295	367	301	176	131
C vs. U_2	C+SB vs. U_2+SB	0.695*	0.417	2107	2365	336	288	157	125
C+SB vs. U_2+SB	C vs. U_2	0.396	0.536	2209	2614	415	324	189	123
	s.e.d	0.104		148		56		23	
	Significance of †:								
	Period	NS		***		*		***	
	Urea	0.1>p>0.05		NS		NS		NS	
	SB	**		NS		NS		NS	

† None of the interactions is significant.

4.5 Discussion

Two experiments were carried out in the previous chapter (Chapter 3) that were designed to shed light on the rules that ruminants might use in making selections between diets of different protein content. It was found that the preference for a food (calculated to be sufficient in eRDP) supplemented with urea, over one that was not, increased as the level of urea increased. The experiment reported here was designed to explore a possible reason for that finding, which was contrary to the expectation that ruminants try to minimise an excess consumption of RDP, at least when supplied as urea (Kyriazakis and Oldham, 1993; Tolkamp *et al.*, 1998b).

The control food used here, C, was similar to the food H of Chapter 3. It was necessary to establish that the effects of urea supplementation on diet choice were similar in the two experiments. In the case of Chapter 3 the proportion of the urea-supplemented food in the diet selected was 0.574 on the lower urea level and 0.667 on the higher. In the current experiment the equivalent figures were 0.466 and 0.593. In the first case the increase is 0.093 and in the second 0.127; both differences were highly significantly different to zero and in good agreement. It happens that in the second case a preference for the food with the lower urea content could not be demonstrated, as the proportion selected of 0.466 was not significantly different to 0.5. However, the general qualitative agreement between the diet compositions selected in the two experiments, strongly suggests that the same effects were operating, whatever they may be.

A possible reason for the excess intake of RDP, seen in both experiments, was raised in the Introduction. On foods of high concentrate content, and with little effective fibre, rumen pH can fall to a level lower than that needed for optimal rumen function (Dougherty *et al.*, 1975; Russell *et al.*, 1979). The ruminant may act to avoid such a low level when given the opportunity (Cooper *et al.*, 1995; Chapter 5). Urea can result in an increase in rumen pH (Kyriazakis and Oldham, 1997; Archimède *et al.*, 1999) and hence, choosing a diet higher in urea might help to overcome the effects of

a reduced pH. The finding in Chapter 3 that sheep chose to eat excess RDP could then be accounted for; they would tolerate the excess RDP in order to get the benefits of the reduced pH.

If the increased preference for foods with higher urea contents is due to their increasing rumen pH then the preference should be able to be changed by using other means to achieve this end. Hay, and the supplementation with $NaHCO_3$, were the means used here. In both cases excess urea may no longer be needed as a buffer and preference for the urea-supplemented food may be reduced, allowing an excess intake of RDP to be avoided, at least to some extent. It is against this background that the results are discussed.

4.5.1 *Single-fed sheep*

Food C was calculated to satisfy the requirements for the eRDP: fME ratio, and for MP requirements of the sheep for potential growth (AFRC, 1993). The addition of urea to this food had no effect on the daily rates of food intake or live weight gain in agreement with Shain *et al.* (1998) and Chapter 3. This is consistent with the presumption of sufficient eRDP in the food (ARFC, 1993). The additional RDP supplied by urea can thus be considered as excess to requirements. Moderate additions of urea to concentrate foods, with adequate eRDP: fME, did not affect food intake and efficiency (Putman *et al.*, 1969; Shain *et al.*, 1994). Large additions of urea (Nomani, 1973), or moderate additions when animals have restricted access to the urea supplemented foods (Bloomfield *et al.*, 1961; Campbell *et al.*, 1963), can reduce either food intake or efficiency. Wilson *et al.* (1975) showed that urea poured into the rumen as a solution two times each day, decreased food intake. The fall in intake was reduced when the same quantity of urea was continuously infused over 24 hours, or was added to the food offered *ad libitum*. It is possible that the sheep in the current experiment coped with the additional RDP from urea, and hence the expected increase in rumen ammonia concentration, by changing their feeding behaviour (Loosli and Warner, 1958; Dulphy *et al.*, 1992). The sheep, with continuous access to

food, may have consumed more frequent, smaller meals that would have allowed the extent of the rise in ammonia concentration to be reduced (Kahn, 1996). This in turn would have reduced the risk of ammonia toxicity (Visek, 1968).

Ha *et al.*, (1983) found that $NaHCO_3$ supplementation caused an increase in the intake of high energy density foods in lambs. This finding is consistent with the evidence reviewed by Erdman (1988). Osbourn *et al.* (1970), and Erdman (1988) proposed that this effect would be because the low rumen pH, found with high energy density foods, would be increased by the addition of $NaHCO_3$. The addition of urea to a food could also be expected to increase the intake of a high energy density food through the same means. It was shown in the *in vitro* test that, in the presence of urease, urea increased the acid buffering capacity of the foods. However, neither $NaHCO_3$ supplementation, nor the addition of urea, increased food intake, which is in agreement with the finding of Hart and Doyle (1985) for $NaHCO_3$. The sheep may have coped with the high energy density food, in the absence of a buffer, by modifying their feeding behaviour as suggested above (Dulphy *et al.*, 1992; Kahn, 1996).

When hay was offered *ad libitum,* along with either the control food or this supplemented with 25g/kg of urea, only trivial amounts of hay were consumed, 28 (s.d. 20) g hay/day in a total food intake of 2356 (s.d. 244) g/day. Surprisingly there was a significant increase in total daily food intake, which, however, was not accompanied by any increase in daily live weight gain. Campion and Leek (1996) showed that as little as 35g of 'fibre' inserted directly into the rumen of sheep increased rumination to over 350 minutes per day compared to 200 minutes per day when 'fibre' was absent. However, the fibre used in Campion and Leek's (1996) experiment was polyethylene that remains unchanged by the digestive processes in the ruminant stomach. The amount of hay needed to achieve a similar level of stimulation of rumination to that achieved by 35g of polyethylene fibre could be expected to be much greater. The hay would need to be reduced in particle size by rumination, be digested and then be removed from the rumen (Bines *et al.*, 1988).

Therefore, it appears unlikely that the, surprisingly significant, increase in daily food intake can be explained as an effect of the trivial amounts of hay consumed. The absence of any increase in intake, when sheep were given hay as part of a choice (treatments 7 to 10, Table 4.2), suggests that the apparent increase in intake observed on treatments 5 and 6 might not have been a real effect, although statistically significant. If the effect is taken to be a real one it is then difficult to account for the increase in intake having no effect on gain and, hence, causing a significant reduction in efficiency.

It is also surprising that the sheep did not consume a greater quantity of hay than they did. Many authors have suggested that ruminants have an 'appetite' for long forage (e.g. Campion and Leek, 1997). Others (e.g. Engku Azahan and Forbes, 1992; Cooper *et al.*, 1996) have found that ruminants may voluntarily include a large proportion of long forage in their diet. One possible explanation for the current results being different is that the amount of concentrate consumed prior to, and during, the experiment maintained the pH of the rumen at a level too low for the hay to be digested (Mould and Ørskov, 1984; Calsamiglia *et al.*, 1999). Prior to the start of the experiment all sheep were offered a high quality pellet without access to long forage. It has been shown that feeding high energy density concentrates can alter the microbial population and result in the loss of cellulolytic bacteria (Erfle *et al.*, 1982). If these bacteria are low in number, then the consumed fibre would not be able to be broken down and digested (Russell and Hespell, 1981). Even though the hay was offered over four weeks, a period that should be sufficient for the cellulolytic bacteria to re-establish (Mould and Ørskov, 1984), the concentrates consumed may have continuously maintained the pH at a level too low for these bacteria to thrive (Russell and Hespell, 1981).

4.5.2 *Choice-fed sheep*

Emmans (1991) suggested that the results obtained from single food treatments can be helpful in interpreting the diet selection made by animals offered food choices

(e.g. Kenney and Black, 1984; Kyriazakis and Oldham, 1993; Cooper *et al.*, 1995; 1996). However, the rules that govern the feeding behaviour of sheep offered a choice will need to be richer than those used to determine the intake of a single food (Arsenos and Kyriazakis, 2001). Sheep may be prepared to consume significant amounts of a food when offered on its own, yet avoid it when offered together with another food as a choice. This was the case in the previous experiment (Chapter 3), where the addition of urea to a single food did not significantly alter food intake in comparison to the control food. However, when offered a choice, the sheep showed a preference for the urea-supplemented foods. If sheep selected the urea-supplemented food in an attempt to maintain rumen pH, the addition of $NaHCO_3$ by reducing the requirement for urea as a buffer, could result in a decrease in the proportion of the diet eaten as the urea-supplemented food. Hay was expected to decrease the risk of a possible disruption of the rumen by the consumption of the control food (Hadjipanayiotou, 1982; Cooper *et al.*, 1996). This could be either directly, as a result of the buffering capacity of the hay (McBurney *et al.*, 1983), or indirectly due to the stimulation of rumination and salivation (Block and Shellenberger, 1980; Campion and Leek, 1997). However, it seems unlikely that the very small amount of hay consumed by the single-fed sheep in the current experiment could have much effect on the pH of the rumen. It would be most unlikely that it would increase to a level such that urea would no longer be needed as a buffer when a choice was offered. The low consumption of hay led to its being offered having no effect on the selection for the foods supplemented with urea.

Overall, when sheep were offered either C and U_1, or C and U_2, with no $NaHCO_3$ supplementation or hay, they selected diets that were in line with those selected in the experiment of Chapter 3. In neither experiment, did the sheep try to minimise an excess consumption of RDP when supplied as urea, which is not consistent with the findings from other experiments (Kyriazakis and Oldham, 1993; Tolkamp *et al.*, 1998b). It was proposed (Chapter 3) that the preference for the urea-supplemented food could reflect its buffering ability (Crawford *et al.*, 1983), which is in agreement with the results of the *in vitro* analysis of the foods used here (Table 4.3). The sheep

may well have been able to distinguish between the post-ingestive consequences of the food with the higher level of urea, and that offered with it as a choice (Parsons *et al.*, 1994; Cooper *et al.*, 1995; Provenza, 1995; Faverdin, 1999; Kyriazakis *et al.*, 1999). They could consequently form a preference for the food supplemented with urea. The addition of only 12.5g urea/kg may not have been enough for any effect to be detected. With no effect detected the sheep would be expected not to form a preference for the urea-supplemented food resulting in a diet selection that was not significantly different from random (0.5), as was the case. The alternative, of course, is that the diet selection of animals on C vs. U_1 choice, were making an informed choice, which happened to be indistinguishable from 0.5.

A supplement of $NaHCO_3$ to both foods, and hence its consumption made unavoidable, significantly decreased the proportion of the urea-supplemented foods in the selected diet. Once an alternative buffer was consumed the need for urea, as a buffer, would be reduced. The food with urea was not totally avoided as the urea had a greater buffering capacity than the $NaHCO_3$ (Table 4.3). The decreased preference for the urea-supplemented food, and hence an avoidance of an excess consumption of RDP, would then be in agreement with the results of Tolkamp *et al.* (1998b). In that experiment, there was a decrease in the preference for the food with the higher eRDP content when urea was added to two forage based foods offered as a choice. Both the sheep in this experiment, and the dairy cows in that of Tolkamp *et al.* (1998b), appear to select to avoid an excess consumption of RDP (Kyriazakis and Oldham, 1993), providing that the source of RDP does not have other properties that are relevant in the test situation.

The results from this and previous experiments, suggest that diets may be selected for their effects, such as buffering capacity (Parsons *et al.*, 1994; Cooper *et al.*, 1995; Kyriazakis *et al.*, 1999; Chapter 5). The supply of such capacity may override other needs, such as the avoidance of an excess intake of RDP. The sheep in this experiment, and those in the previous ones, were prepared to over consume RDP supplied by urea. By supplying $NaHCO_3$, the need for urea as a buffer was reduced.

This led to a decrease in the proportion of the urea supplemented foods in the diets selected, and hence to a reduction in the excess consumption of RDP.

Ruminants in more complex situations, such as those found in grazing and natural systems, have to make complex foraging decisions such as distinguishing between plant species within the sward, as well as between plant parts within a species. This enables them to select a diet that may meet their nutrient requirements (Illius *et al.*, 1992). Simple experiments such as that reported here are a necessary first step in understanding and predicting the diet selection of ruminants in more complex situation as theories of diet selection seek to be general. Accounting for the behaviour of the animals even in such simple cases is by no means straightforward as has been shown.

Chapter 5

The effect of consumption of foods that differ in energy density and/or sodium bicarbonate supplementation on subsequent diet selection in sheep

Arriving at one goal is the starting point to another.
John Dewey (1859-1952)

5.1 Abstract

The short-term consumption of foods that differed in energy density (ED) and/or sodium bicarbonate ($NaHCO_3$) supplementation, on subsequent food intake and diet selection in sheep were measured. Thirty sheep weighing 35.9 (S.D. 2.89) kg were used. Two foods were formulated: H had 11 and L had 8 MJ ME/kg fresh matter. Four further foods were formulated by adding either 40g $NaHCO_3$/kg or 16.5g NaCl/kg to foods H and L. Sodium chloride was added to give the same Na concentration as with 40g $NaHCO_3$/kg to control for any effects of Na *per se*. In a preliminary test, it was found that a 2 h consumption of H supplemented with $NaHCO_3$ could buffer potential impact on the rumen environment of subsequent consumption of food H alone (as judged by rumen pH and acid buffering capacity); however, it was not as effective as the consumption of food L alone in doing so. Each food treatment was offered to one of six groups (n=5) for 2 h following 16 h of food deprivation. Sheep were then offered a choice between H and L for a further 6 h. Supplementing H or L with either $NaHCO_3$ or NaCl had no significant effect on either intake or diet selection. Energy density significantly ($p<0.01$) affected intake during the 2 h single feeding period, with sheep offered H or L consuming 540 and 663 (s.e.d. 37) g respectively, but had no effect on subsequent intake during the choice period. During the choice period all sheep showed a preference for food H, but sheep previously offered L selected significantly more H (0.873 kg/kg) than sheep previously offered H (0.544 (s.e.d. 0.028) kg/kg; $p<0.001$). It is concluded that short-term consumption of foods that differ in ED, and hence in their potential impact on the rumen environment, significantly affect subsequent diet selection. This is in agreement with the hypothesis that ruminants select a diet to help maintain the rumen environment within a certain physiological range. Forty grams $NaHCO_3$/kg added to H may not have been sufficient to affect subsequent diet selection. It is suggested that larger, rather than smaller, changes in the rumen environment achieved through previous feeding, should be expected to alter subsequent diet selection.

5.2 Introduction

Grazing animals make complex foraging decisions including distinguishing between plant species within the sward and between plant parts within a species. Making such distinctions enables them to select a diet that may meet their nutrient and energy requirements (Illius *et al.*, 1992). It has been suggested by many authors (e.g. Kenney & Black, 1984; Van Wieren, 1996), and is assumed in foraging models based on the idea of optimality (Charnov, 1975; Belovsky, 1978; Belovsky & Schmitz, 1994), that animals select a diet that maximizes their rate of energy intake. However, ruminants have clearly been seen not to maximize their short-term energy intake rates whilst grazing (Newman *et al.*, 1994; Parsons *et al.*, 1994). In addition, sheep offered a choice between two concentrate foods that differed in their energy density (ED) included a considerable proportion of the low ED food in their diet (Cooper *et al.*, 1995, 1996). These results appear to contradict the energy rate maximization assumption of the optimal foraging theory (Stephens & Krebs, 1986).

The potentially disruptive effects on the rumen environment, such as a fall in pH, caused by the consumption of a high ED food that is readily fermentable (Kaufman, 1976), may account for ruminants not foraging in accordance with the optimal foraging theory (Parsons *et al.*, 1994; Cooper *et al.*, 1995, 1996; Kyriazakis *et al.*, 1999). The maintenance of pH within the rumen is of great importance to resident micro-organisms (Russell *et al.*, 1979) and is of importance to the host (Owens *et al.*, 1998). It has thus been suggested that one of the objectives of the diet selection of ruminant animals might well be to maintain a fit and adaptive rumen (Cooper *et al.*, 1995, 1996; Faverdin, 1999). It could be hypothesized that ruminants include a large proportion of a low ED food in their diet in order to dilute the disruptive effects of a high ED food when they are given a choice. However, including some of the lower ED food into the diet could result in a lower energy intake than when the diet consists solely of the high ED food (Kyriazakis & Oldham, 1993; Cooper *et al.*, 1995). Ruminants can thus be seen as making a trade-off between the potential

benefits of selecting a higher energy food against the potential cost of disrupting the rumen environment, when having to select their diet from two foods that have different effects on the rumen environment (Kyriazakis *et al.*, 1999). Short-term modifications in diet selection could be seen as aiming firstly, to prevent further disruption to the rumen environment after a 'disruptive' food has already been consumed, and secondly, to return the conditions in the rumen to within an acceptable range as soon as possible.

The objective of this experiment was to determine the effect of short-term consumption of foods that differ in ED and/or $NaHCO_3$ supplementation on subsequent food intake and diet selection in sheep. We hypothesized that the extent of subsequent selection for the low ED food, when sheep are offered a choice between a high and low energy food, would depend on prior consumption of such foods. We expected that animals that have consumed a high ED food would select a diet with a higher proportion of the low ED food and vice versa. Addition of $NaHCO_3$, which has the potential to act as a buffer to the high ED food consumed singly, to the food consumed previously, would be expected to reverse such diet selection.

5.3 Materials and Methods

5.3.1 Animals and Housing

Thirty Texel x Greyface wether sheep, approximately one year of age and weighing 35.9 (S.D. 2.89) kg at the start of the experiment were used. They had previously been housed as groups and fed hay of medium quality *ad libitum*. They were kept in individual, raised pens (2 x 1.5m) in a shed that was naturally ventilated. Throughout the experiment artificial lighting was provided from 0600 to 1800 h but, as the experiment was carried out during March to May 1998 at latitude 56°N, the duration of daylight had the potential to be longer than the period of artificial lighting. The sheep were given 21 days acclimatization to allow them to become accustomed to their new environment and experimental procedures before the start of the experiment. They were offered *ad libitum* a high quality pelleted food with 184g crude protein (CP)/kg dry matter (DM) and 10 MJ metabolisable energy (ME)/kg DM with no access to long forage. All sheep had free and continuous access to water throughout.

5.3.2 Experimental Foods

Two basal foods, H and L, that differed in ED were formulated (Table 5.1). Food H was designed to at least meet the metabolisable protein (MP) and ME requirements of sheep of 36kg live weight for potential growth (Agricultural and Food Research Council (AFRC), 1993). Food L was designed to be deficient in energy content to support potential growth when offered alone on an *ad libitum* basis. Food L was made by diluting H with oatfeed, a much less digestible material. It was expected that L would be fermented more slowly than H (Ministry of Agriculture, Fisheries and Food (MAFF), 1990) and therefore would be potentially less disruptive to the rumen environment. The MP:ME, eRDP:fME (effective rumen degradable protein and fermentable ME respectively) and minerals:ME ratios were kept as similar as possible in L and H. This was done in order that the selection made by sheep offered

Table 5.1. *Ingredients and determined chemical analyses of the two basal experimental foods. The calculated yields of ME and protein components are also given.*

	Foods	
	H	L
Ingredients (g/kg)		
Barley	411	120
Oatfeed	40	572
Citrus Pulp	300	71
Soya Bean Meal (solvent extracted)	155	148
50% Fat Premix†	25	25
Molasses	50	50
Salt	6.8	4.0
Dicalcium Phosphate	3.5	1.2
Limestone Flour	6.0	5.9
Calcified Magnesite	0.1	0.4
Vitamin and Mineral Mix‡	2.5	2.5
Total	1000	1000
Chemical Analysis (g/kg DM)		
Dry Matter (g/kg)	874	887
Crude Protein	158	117
Crude Fibre	89	185
Modified Acid Detergent Fibre	137	278
Neutral Detergent Fibre	226	475
Ash	71	69
Calcium	9.3	6.6
Phosphorus	4.0	2.9
Sodium	3.4	2.5
Sulphur	1.9	1.8
Estimated yields§		
Metabolisable Energy (ME; MJ/kg DM)‖	12.7	9.0
Fermentable ME (fME; MJ/kg DM) ‖	11.7	8.0
Metabolisable Protein (MP; g/kg DM)	101.9	74.5
eRDP (g/kg DM)¶	114.3	80.0
MP:ME (g/MJ)	8.0	8.3
eRDP:fME (g/MJ)	9.8	10.0

† Manufactured and supplied by Central Farmers Ltd, Methil, Fife, Scotland.

‡ Vitamin and Mineral mix used was Scotmin ewe/lamb (Scotmin Nutrition Ltd, Ayr, Scotland).

§ Values calculated using the metabolisable protein system (AFRC, 1993) assuming rumen outflow rate of $0.05h^{-1}$ and standard values for degradability coefficients (MAFF, 1990).

‖ ME and fME, calculated from food tables (MAFF, 1990)

¶ eRDP, effective rumen degradable protein.

a choice between the two foods could be interpreted on an energy content basis (Cooper *et al.*, 1994).

Five additional foods were formulated by adding 10, 20, 40 or 80g $NaHCO_3$/kg fresh matter to H (H_{10}, H_{20}, H_{40} and H_{80} respectively) and 40g $NaHCO_3$/kg fresh matter to L (L_{40}). Sodium bicarbonate was selected for this experiment because many studies have previously shown that $NaHCO_3$ is an effective dietary buffer for ruminants when added to foods of low fibre and high energy content (Ha *et al.*, 1983; Hart & Polan, 1984). It was expected that $NaHCO_3$ would have beneficial effects on the rumen environment by reducing the decline in pH when H was consumed. Two further foods were formulated by adding 16.5g NaCl/kg fresh matter to H and L (H_{NaCl} and L_{NaCl} respectively). Sodium Chloride was added to both foods to provide the same concentration of Na that would result from adding 40g $NaHCO_3$/kg, to control for the effects of Na *per se* (Carter & Grovum, 1990).

5.3.3 Experimental Design

5.3.3.1 Test A

The two foods used in this two week test were the two basal foods H and L. The sheep were allocated to one of two groups (n=15), taking into account their live weight at this point, so that the mean live weight of each group was similar (35.9 (S.D. 1.68) kg). One group was offered a choice between foods L and H from 0900 to 1700 h daily. The other group was offered the same choice of foods continuously. The positions of the two foods within a pen were randomized within group, but their positions were not changed during the testing period.

The test was performed to determine (i) whether sheep in our experimental conditions do select between a high and low ED food in a manner consistent with the idea of a trade-off and include substantial amounts of food L in their diet, and, (ii) the effect of offering foods for a period of 8 h instead of 24 h daily on diet selection between two foods that differed in ED. An 8 h feeding period was required to

increase feeding motivation in Tests B and C to ensure that the treatment foods offered were consumed (see below).

5.3.3.2 Test B

The six foods used in this test were the two basal foods, H and L, and food H supplemented with $NaHCO_3$ (H_{10}, H_{20}, H_{40} and H_{80}). The test lasted for a period of 11 days. Sheep were allocated to one of six groups (n=5) in accordance to their live weight at this point, so that the mean live weight of each group at the beginning of the test were similar (40.6 (S.D. 2.88) kg). Groups 1 to 6 were offered one of: H, H_{10}, H_{20}, H_{40}, H_{80} or L, the treatment foods, for a period of 2 h from 0900 to 1100 h. At 1100 h all refusals were removed and weighed and all sheep were offered food H alone until 1700 h, when all foods were removed.

This test was carried out to determine how disruptive the two ED foods used in this experiment were on the rumen environment after they had been offered for 2 h and what concentration of $NaHCO_3$ should be added to food H to obtain the most beneficial combined effects on rumen pH and intake, when offered for a 2 h period. It has been demonstrated that the buffering effects of $NaHCO_3$ added to a food are greatest 2 to 8 h after initial consumption (Erdman, 1988). For this reason the foods containing the $NaHCO_3$, along with foods H and L for comparison, were offered for a period of 2 h, the minimum time suggested before the effects of $NaHCO_3$ supplementation were greatest. Measurements of subsequent intake were collected for a further 6 h after the treatment foods had been removed.

5.3.3.2.1 Sample processing and analysis of the rumen samples. Rumen samples were collected via a stomach tube every 2 h from 0900 to 1700 h inclusively on every alternate day starting on day 1. The 2 h sampling times were selected as these coincided with the measurements collected on food intake. A 2 h collection period was also relevant to pH as it was predicted that the pH of the rumen environment at 1100 h, when treatment foods were removed, would influence subsequent intake. Each sheep within a group was sampled once each day but the time of sampling

changed on each of the sampling days. This resulted in each sheep being sampled at each of the sampling times by the end of the test, with the sequence of the samples on day 1 being repeated on day 11.

The pH of the rumen samples was measured immediately after collection using a glass electrode (Model RL 250/pH/ISE meter, Russell Laboratories Ltd., Auchtermuchty). The rumen samples were then strained through double-thickness muslin and treated with 250µl saturated mercuric chloride prior to freezing for subsequent analysis of ammonia nitrogen (NH_3-N) concentration using an ion-selective electrode (Model 95-5129, Russell Laboratories Ltd, Auchtermuchty).

The *in vitro* acid buffering capacity of the strained rumen samples was measured using a modification of the procedure by Jasaitis *et al.* (1987). A 5ml rumen sample was suspended in 50ml distilled deionized water and stirred continuously with a magnetic stir bar. Titrations were performed by the addition of acid (0.1 N HCl) until the pH was decreased to 4. Acid buffering capacity was calculated by dividing titratable acidity (total volume of acid added to each sample multiplied by its normality) by the total change in concentration of hydrogen ions ($[H^+] = 10 \times (\exp(-pH))$). The base buffering capacity of the rumen samples was not measured in this experiment as the rumen pH seldom exceeds pH 7, especially when high energy, readily fermentable concentrate foods are offered (Erdman, 1988).

5.3.3.3 Test C

The six foods used in this test were the two basal foods, H and L, these foods supplemented with either 40g $NaHCO_3$/kg (H_{40} and L_{40}) or with 16.5g NaCl/kg (H_{NaCl} and L_{NaCl}). Test C lasted for a period of 21 days, a length of time expected to be sufficient for any pattern in subsequent diet selection to be observed (Cooper *et al.*, 1996). Sheep were allocated to one of six groups (n=5) by live weights of each group at the beginning of the test were similar (41.2 (S.D. 1.36) kg). Groups 1 to 6 were offered one of: H, H_{40}, H_{NaCl}, L, L_{40}, or L_{NaCl} for a period of 2 h from 0900 to 1100 h. At 1100 h all refusals were removed and weighed and all sheep were offered H and L

as a choice until 1700 h, when all foods were removed. The position of the two foods within a pen, offered as a choice, were randomized within treatment. The position was not changed during the testing period. When only one food was offered the food trough was in a position central to the choice positions. This test was designed to address the main objective of the experiment which was to determine the effect of consumption of foods that differ in ED and/or $NaHCO_3$ supplementation on subsequent diet selection in sheep.

5.3.4 Measurements

The weight of all food troughs containing fresh food were recorded at 0900 h and offered to the sheep each day. At 2 h intervals, from 0900 to 1700 h inclusively, food intake was recorded by removing the food troughs, weighing them with their contents and returning them to the pens. The amount consumed was then calculated by subtracting the weight of the trough with its contents from the previous weight recorded. The live weights of the sheep were measured on the first day of each test, which were conducted consecutively, then weekly thereafter.

Samples of all foods offered were taken every week and a composite sample was analyzed for DM, CP, neutral detergent (plus amylase) fibre (NDF), acid detergent fibre (ADF), sodium, calcium and phosphorus contents as described by MAFF (1993), (Table 5.1).

5.3.5 Statistical Analysis

All data were analyzed using GENSTAT for Windows, version 5.2 (Lawes Agricultural Trust, 1993) unless otherwise stated. The diet selection data (expressed as the proportion of food intake taken as food H) were normally distributed and were not transformed for further analysis. Where appropriate, a Students 't'-test with a null hypothesis of mean = 0.5 for each group was used to test whether the proportion of food selected differed from random (Minitab for Windows release 11.1, Minitab Inc., 1996).

5.3.5.1 Test A

The data for total food intake (TFI) and diet selection for each 2 h interval, and daily, were analyzed using one way analysis of variance. To determine whether there was a time interval effect on intake and diet selection, data from each time interval for each group from 0900 to 1700 h, were treated as repeated measures and analyzed as a split plot design where time interval was the sub-plot factor, nested within sheep. For each sheep the linear regression coefficient for weight on time, over the two weeks was used to estimate live weight gain. The rate of gain and food conversion efficiency (FCE; g of weight gained/kg food eaten) were analyzed using one way analysis of variance.

5.3.5.2 Test B

The data for TFI for each individual 2 h interval and daily (0900-1700 h) were analyzed using one way analysis of variance. Data collected from rumen samples were analyzed using Residual Maximum Likelihood (REML) due to the unbalanced nature of the data. Wald tests on pH, NH_3-N and acid buffering capacity are obtained from the REML procedure and were used to determine any significant differences present using the degrees of freedom and probability value for the fixed effect (group) provided by REML (Lawes Agricultural Trust, 1993).

5.3.5.3 Test C

TFI and diet selection data for each 2 h interval, over the choice period, and daily where appropriate, were analyzed as a 2x3 factorial design using analysis of variance. Effects of, and interactions between ED (H or L) and supplement (none, $NaHCO_3$ or NaCl) were analyzed with ED and supplement as factors. These data for each group, over the choice period (1100 to 1700 h), were also subjected to repeated measures analysis as a split plot design (see Test A) to determine whether there was an effect of time on food intake and diet selection. For each sheep the linear regression coefficient for weight on time, over the three weeks was used to estimate live weight gain. The rate of gain and FCE were analyzed as a 2x3 factorial design as described above.

5.4 Results

5.4.1 Test A

Results for Test A are given in Table 5.2. TFI was significantly greater ($p<0.05$) for sheep offered the foods for 24 h daily. However, sheep offered food for 8 h daily consumed significantly more ($p<0.001$) during 3, out of the 4, 2 h intervals than those offered foods for 24 h. Daily food intake significantly ($p<0.001$) increased from week 1 to week 2 of the test for both groups with no interaction between week and group. There was a significant effect of time interval ($p<0.05$) on the amount of food consumed. The amount of food consumed by the sheep offered food 8 h daily was significantly greater ($p<0.05$) between 0900 and 1100 h than that consumed during any other 2 h period; the amount consumed during the following 2 h period (1100 to 1300 h) was significantly less ($p<0.05$) than that consumed during any other 2 h period. The only time interval effect on food consumption between 0900 and 1700 h by sheep offered food 24 h daily was that the amount consumed between 1100 to 1300 h, which was significantly less ($p<0.05$) than that consumed between 0900 to 1100 and 1500 to 1700 h.

The overall proportion of food H in the diet selected was significantly greater ($p<0.05$) for the sheep offered food 24 h daily and it increased significantly ($p<0.001$) from week 1 to week 2 for both groups. The proportion of food H selected from 0900 to 1500 h was consistently higher for sheep offered food 24 h daily, though not significant. However, during 1500 to 1700 h the difference in the proportion of food H selected by the two groups was significant ($p<0.05$). There was no effect of time interval on the proportion of food H selected by either of the two groups.

Intake of ME differed significantly ($p<0.01$) between groups: 14.22 and 16.53 (s.e.d. 0.660) MJ/d for sheep offered food 8 and 24 h daily respectively. Similarly, live weight gain ($p<0.01$) and FCE ($p<0.05$) differed significantly between groups: 291 and 397 (s.e.d. 31.7) g/d and 188 and 233 (s.e.d. 18.8) g gained/kg food for sheep offered food 8 and 24 h daily respectively.

Table 5.2. *Total food intake and the proportion of H selected (kg/kg) during each 2 h interval and daily, and the daily ME intake of sheep offered a choice between 2 foods that differed in energy density (H; 11MJ ME/kg and L; 8MJ ME/kg) either for a period of 8 or 24 h/day over a two week period (Test A). Asterisks (*) next to the proportion of H selected denote that the diet selected differs significantly from random*

	Food availability			
	8 h	24 h	s.e.d	significance
Total food intake (g)				
0900-1100 h	683	208	32.3	***
1100-1300 h	192	166	14.4	NS
1300-1500 h	315	196	12.6	***
1500-1700 h	365	228	18.0	***
1700-0900 h	na	930	na	na
s.e.d.	24.4	16.8		
Daily food intake (g/d)	1555	1728	75.6	*
Proportion of H selected (kg/kg)				
0900-1100 h	0.357*	0.452	0.071	NS
1100-1300 h	0.429	0.528	0.079	NS
1300-1500 h	0.417*	0.528	0.070	NS
1500-1700 h	0.407*	0.543	0.068	*
1700-0900 h	na	0.573	na	na
s.e.d.	0.056	0.085		
Daily proportion of H selected (kg/kg)	0.387*	0.536	0.069	*

s.e.d., standard error of the difference of the means; na, not applicable; NS, not significant; *, $p<0.05$; ***, $p<0.001$.

TFI of the treatment foods (0900 to 1100 h), food H (1100 to 1700 h) and daily food intake (0900 to 1700 h) for each of the 6 groups of sheep offered different treatment foods in Test B are shown in Table 5.3. There was a dose response to the supplementation of $NaHCO_3$ to food H on food intake during the 2 h period that the treatment foods were offered (0900 to 1100 h). This response was significantly quadratic ($p<0.05$). Intake of sheep offered H, supplemented or unsupplemented, was significantly ($p<0.01$) lower than that of sheep offered L during the 2 h period.

As the amount of $NaHCO_3$ increased from 0 to 80g/kg in the treatment foods offered from 0900 to 1100 h, the subsequent amount of the H food consumed from 1100 to 1700 h also increased. This increase was significant and essentially linear ($p<0.01$) for every 2 h period interval. Sheep offered H as a treatment food had a significantly lower intake between 1100 to 1700 h as well as daily, than any other group. This resulted in their ME intakes being significantly lower than that of any other group. Sheep offered food L as a treatment food consumed significantly more daily than any other group and had a significantly greater ME intake (Table 5.3).

The pH and acid buffering capacity of rumen samples collected at each 2 h interval from 0900 to 1700 h inclusively, for each of the 6 groups of sheep offered different treatment foods in Test B are shown in Table 5.4. As the amount of $NaHCO_3$ supplementation increased from 0 to 80g/kg in the treatment foods offered from 0900 to 1100 h, the pH of rumen samples collected at 1100 h also increased. This increase was significant and essentially linear ($p<0.001$). The consumption of food L during 0900 to 1100 h did not result in a pH decline to the same extent as that recorded from sheep that were offered food H from 0900 to 1100 h. The pH recorded during all subsequent sampling times (1300, 1500 and 1700 h) did not differ significantly between groups. There was no effect of the treatment food offered from 0900 to 1100 h, or sample time on the acid buffering capacity of the rumen samples. However, the acid buffering capacity of food H from 1100 h onwards was always lower than that

Table 5.3. *Total food intake during each 2 h interval and daily, and the daily ME intake of sheep offered one of the basal foods (H; 11MJ/kg or L; 8MJ/kg) of basal food H supplemented with 10 (H_{10}), 20 (H_{20}), 40 (H_{40}) or 80 (H_{80}) g $NaHCO_3$/kg between 0900 and 1100 h followed by food H unsupplemented for a further 6 h (Test B). Asterisks (*) denote that values within a row that differ significantly for the intake of sheep offered the H treatment food unsupplemented.*

	Treatment							
	H	H_{10}	H_{20}	H_{40}	H_{80}	L	s.e.d.	significance
Treatment food (g)								
0900-1100 h	305	399	409	398	317	769*	72.5	***
H food (g)								
1100-1300 h	131	160	253*	242	329*	306*	56.8	*
1300-1500 h	134	207*	223*	234*	245*	262*	26.5	**
1500-1700 h	158	242*	263*	244*	289*	317*	30.1	***
1100-1700 h	423	609*	739*	720*	862*	885*	88.8	***
Daily food intake (g/d)	728	1007*	1148*	1118*	1179*	1654*	106.6	***
Daily ME intake (MJ/d)	8.0	11.1*	12.6*	12.3*	13.0*	15.9*	1.13	***

s.e.d., standard error of the difference of the means; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Table 5.4. *The pH and acid buffering capacity (ABC) of rumen samples collected at each 2 h interval from 0900 to 1700 h inclusively, from sheep offered one of the basal foods (H; 11MJ/kg or L; 8MJ/kg) or basal food H supplemented with 10 (H_{10}), 20 (H_{20}), 40 (H_{40}) or 80 (H_{80}) g $NaHCO_3$/kg between 0900 and 1100 h followed by food H unsupplemented for a further 6 h (Test B).*

Treatment	Sampling time									
	0900 h		1100 h		1300 h		1500 h		1700 h	
	pH	ABC (eq) †	pH	ABC (eq)	pH	ABC (eq)	pH	ABC (eq)	pH	ABC (eq)
H	7.18	3.53	5.28	2.96	5.54	2.55	5.33	2.77	5.37	1.91
H_{10}	7.51	3.75	5.35	2.76	5.62	2.88	5.47	3.51	5.33	3.84
H_{20}	7.26	3.91	5.70	2.84	5.54	3.09	5.58	2.92	5.28	3.58
H_{40}	7.04	3.63	5.77	3.01	5.63	3.16	5.58	3.41	5.27	3.31
H_{80}	7.22	3.99	6.16	2.84	5.69	3.61	5.59	3.15	5.20	2.79
L	7.21	3.26	6.05	3.53	5.95	3.56	5.90	3.55	5.36	3.09
mean	7.24	3.68	5.72	2.99	5.66	3.14	5.58	3.22	5.30	3.09
s.e.d.	0.20	1.95	0.26	1.45	0.25	1.45	0.32	1.54	0.16	1.76
significance	NS	NS	**	NS	NS	NS	NS	NS	NS	NS

†, Equivalents (eq) of HCl required to lower one unit of [H+] of 5ml rumen liquor suspended in 50ml distilled deionized water to 10^{-4} (pH 4), divided by total in [H+] change (initial concentration minus 10^{-4} (pH 4)).
s.e.d., standard error of the difference of the means; NS, not significant; **, $p<0.01$.

measured from sheep offered H supplemented with 40g $NaHCO_3$/kg as a treatment food. Sheep offered H supplemented with 40g $NaHCO_3$/kg in turn had a lower acid buffering capacity at sampling times 1100, 1300 and 1500 h than that measured from sheep offered food L as a treatment food from 0900 to 1100 h.

NH_3-N concentrations measured at any sampling time did not differ significantly between groups. The concentration of NH_3-N did however decrease significantly from the first sample time to the second for all groups (mean NH_3-N concentration at 0900 and 1100 h was 192 and 136 (s.e.d. 12.6) mg/l respectively). There was no further effect of time on NH_3-N concentration.

Combining the effects of $NaHCO_3$ supplementation on food intake between 0900 and 1100 h when these foods were offered with the pH and acid buffering capacity at 1100 h when treatment foods were removed, 40g $NaHCO_3$/kg was superior to all other $NaHCO_3$ concentrations and hence used for Test C.

5.4.3 Test C

Total food intakes during each 2 h interval and daily are given in Table 5.5. ED had a significant effect on intake between 0900 and 1100 h. Overall, sheep offered the L basal food consumed significantly ($p<0.01$) more than sheep offered the H basal food during this time period. There was no significant effect of $NaHCO_3$ or NaCl supplementation on food intake nor were there any significant interactions between ED and supplementation on food intake between 0900 and 1100 h. Neither ED nor $NaHCO_3$ nor NaCl supplementation had a significant effect on the subsequent TFI between 1100 and 1700 h. Daily food intake did not differ significantly between groups.

Sodium bicarbonate or NaCl additions to foods offered between 0900 and 1100 h had no significant effect on subsequent diet selection irrespective of the food to which

they were added. Data for groups offered the same basal food, irrespective of supplementation between 0900 and 1100 h, were pooled for subsequent diet selection analysis. Fig. 1 shows the mean proportion of food H selected from 1100 to 1700 h by sheep initially offered H or L between 0900 and 1100 h. Overall, the preference for the H food was significantly greater in sheep initially offered L than in sheep initially offered H ($p<0.001$). This difference was significant in all of the 2 h time intervals considered, but its size diminished with time. The mean proportion of H selected from 1100 to 1700 h was 0.544 and 0.873 (s.e.d. 0.0280) kg H/kg TFI for sheep previously offered food H or food L between 0900 to 1100 h respectively.

There was no significant effect of previous food offered, from 0900 to 1100 h, on live weight gain or FCE. The live weight gains and FCE for sheep initially offered H or L between 0900 and 1100 h were 258 and 293 (s.e.d. 18.2) g/d and 158 and 163 (s.e.d. 8.5) g gained/kg food respectively.

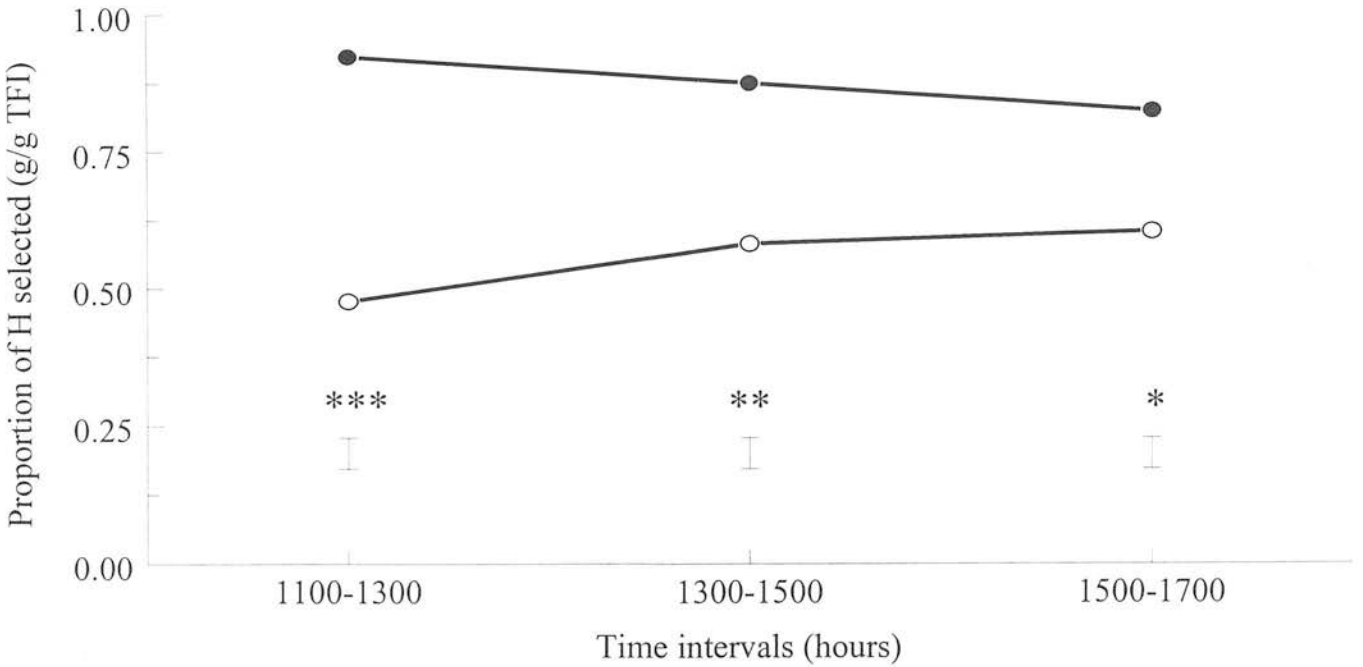
Table 5.5. *Total food intake during each 2 h interval and daily, and the daily ME intake of sheep offered one of the basal foods (H; 11 MJ ME/kg or L; 8MJ ME/kg) or one of these basal foods supplemented with either 40g $NaHCO_3$/kg (H_{40}, L_{40}) or 16.5g NaCl/kg (H_{NaCl}, L_{NaCl}) for a period of 2 h from 0900 to 1100 h followed by a choice between the basal foods H and L from 1100 to 1700 h (Test C).*

	Treatment							Significance of:	
	H	H_{40}	H_{NaCl}	L	L_{40}	L_{NaCl}	s.e.d	ED	supplementation[†]
Treatment food (g)									
0900-1100 h	539	610	472	718	663	607	59.2	**	NS
Choice period (g)									
1100-1300 h	342	384	471	440	511	469	74.8	NS	NS
1300-1500 h	299	327	363	315	293	287	39.9	NS	NS
1500-1700 h	329	366	446	393	362	361	59.1	NS	NS
1100-1700 h	970	1077	1280	1147	1166	1117	146.1	NS	NS
Daily Food Intake (g/d)	1509	1687	1751	1865	1829	1724	168.7	NS	NS
Daily ME intake (MJ/d)	15.3	17.2	17.3	17.5	17.6	16.7	1.50	NS	NS

[†] The interaction between ED and supplementation was not significant.

ED, energy density; s.e.d., standard error of the difference of the means; NS, not significant; **, $p<0.01$.

Figure 5.1. *The proportion of H selected (kg/kg) by sheep offered a choice between two foods that differ mainly in energy density (H, 11MJ ME/kg and L, 8MJ ME/kg) from 1100 to 1700 h. The sheep had previously been offered one of H, ❍ or L, ● alone for a period of 2 h immediately prior to the choice of foods being offered. (*, p<0.05; **, p<0.01; ***, p<0.001).*



5.5 Discussion

Diets selected by ruminants in a number of previous experiments (e.g. Newman *et al.*, 1994; Parsons *et al.*, 1994; Cooper *et al.*, 1995, 1996; Concha & Nicol, 2000) have not been consistent with the assumption of the optimal foraging theory, as the animals did not maximize their short-term rate of energy intake. The current experiment was expected to provide some insight into why ruminants, in the short-term, select a diet with a lower ME content than available.

It has been suggested that ruminants might select a diet in an attempt to maintain their rumen environment within a certain physiological range (Parsons *et al.*, 1994; Cooper *et al.*, 1995, 1996). It could therefore be hypothesized that ruminants include a large proportion of the lower ED food in their diet, and hence do not maximize their short-term energy intake rates, in order to dilute the disruptive effects of the high ED food when they are given a choice. Ruminants can be seen as having to make a trade-off in their diet selection. The benefits of selecting a higher ED food, and hence meeting their energy requirements, need to be set against the cost of disrupting the rumen environment (Kyriazakis *et al.*, 1999). Short-term modifications in diet selection could therefore be seen as aiming firstly, to prevent further disruption to the rumen environment after a 'disruptive' food has already been consumed, and secondly, to return the conditions in the rumen to within an acceptable range as soon as possible (Kyriazakis, 1997).

The objective of this experiment was to determine the effect of consumption of foods that differ in ED and/or $NaHCO_3$ supplementation, hence differing in their potential effects on the rumen environment, on subsequent diet selection in sheep. Two important preliminary tests (A and B) were needed before moving to the main objective.

5.5.1 Test A

During this test sheep offered a choice of foods for 24 h daily did not predominantly select the high ED food as would have been predicted by the optimal foraging theory (Stephens & Krebs, 1986), but selected a diet that did not differ significantly from random and which had a considerable proportion of L. Restricting the time that food was offered to 8 h daily produced a non-random diet selection with the sheep showing a marked preference for L. The proportion of H in the diet selected increased from the first week to the second week of this test for both groups. The increase in the proportion of H selected could have been due to the adaptation of the ruminant to the products of fermentation associated with the foods offered and the adaptation of the microbes to both the foods offered and the new rumen environment (Mackie *et al.*, 1978).

The results from this test contradict the rate maximization assumption of the optimal foraging theory (Stephens & Krebs, 1986). They are in agreement with the hypothesis that sheep select a diet consistent with the idea of a trade-off and balance the benefits of selecting the higher ED food against the cost of disrupting the rumen environment (Cooper *et al.*, 1995, 1996; Faverdin, 1999; Kyriazakis *et al.*, 1999). The direction of the change in diet selection, as time availability of the foods offered as a choice was reduced, is particularly in support of this.

5.5.2 Test B

Sheep on H, which was used as a treatment food from 0900 to 1100 h had a significantly lower intake than any other group of sheep. At 1100 h this group of sheep also had the lowest recorded rumen pH. This suggests that the H food was indeed disruptive to the rumen environment and could potentially cause sheep to limit their intake of this food and increase the intake of an alternative food when given a choice (Phy & Provenza, 1998 a, b). The qualities of food H therefore met the requirements of this experiment.

Osbourn *et al.* (1970) and Erdman (1988) proposed that supplementing a high ED food with $NaHCO_3$ would increase food intake through an increase in rumen pH. This was found to be the case during the 2 h period that the treatment food was offered, with the exception of sheep offered foods supplemented with 80g $NaHCO_3$/kg. However, mineral salts cause an increase in osmolality when consumed (Hart & Polan, 1984), which in turn has been demonstrated to have an adverse effect on intake (Carter & Grovum, 1988, 1990). The reduced food intake of sheep on the treatment supplemented with 80g $NaHCO_3$/kg can thus be explained by an increased osmolality effect as proposed by Carter and Grovum (1988, 1990).

The addition of 40g $NaHCO_3$/kg was superior to all other $NaHCO_3$ concentrations as judged by the combined effects on food intake, pH and acid buffering capacity at 1100 h and hence used for Test C. It can, however, be seen that 40g $NaHCO_3$/kg was not as effective as L in decreasing the fall in rumen pH and increasing subsequent intake of food H. Sheep offered food L for 2 h managed to consume subsequently significantly more H food and significantly more ME daily, than any other group. This suggests that the magnitude of disruption was less for sheep offered food L and that the beneficial effects of food L on the rumen environment were carried over for a longer period than those of any other treatment.

5.5.3 Test C

It was hypothesized that ruminants would select a diet as a result of a trade-off between the benefits of maximizing energy intake against the costs of disrupting the rumen environment (Parsons *et al.*, 1994; Cooper *et al.*, 1995, 1996). The objective of Test C was to determine the effect of consumption of foods that differ in ED and/or $NaHCO_3$ on subsequent diet selection in sheep. It was expected that sheep initially offered the disruptive H food for a short period of time would need to include a larger proportion of L in their subsequent diet to return conditions in the rumen to within an acceptable range as soon as possible. However, sheep initially offered the L food would not have experienced the same disruption to the rumen

environment and therefore would not require as large a proportion of food L in their subsequent diet resulting in an increased ME intake. During Test B $NaHCO_3$ supplementation had reduced the disruptive effects of the pre-offered H food by reducing the fall in rumen pH (Ha *et al.*, 1983; Hart & Polan, 1984). However, 40g $NaHCO_3$/kg was not as effective as food L in reducing the disruption of the rumen environment. Therefore, it was expected that the subsequent proportion of food L selected to reduce the disruption caused by the pre-offered, H food supplemented with $NaHCO_3$ would lie between that selected by sheep offered either H or L unsupplemented as a treatment food.

In agreement with the above hypothesis, sheep initially offered the disruptive H food selected a greater proportion of L during the subsequent period compared to those initially offered L. It was found during Test B that the consumption of food H resulted in a low rumen pH and a decrease in subsequent food intake. Therefore, it is likely that sheep initially offered H needed to include a large proportion of L in their diet when offered a choice. On the other hand, food L, when consumed alone did not disrupt the rumen to the same degree as food H (Test B) due to its predicted slow fermentation rate. Therefore food L would dilute the subsequent disruptive effects of the H food as it was consumed and buffer the by-products of fermentation (McBurney *et al.*, 1983; Jasaitis *et al.*, 1987). As the rumen environment was not disrupted to a large degree, a low proportion of L was required during the subsequent diet selection resulting in an increased ME intake.

Supplementing food H with 40g $NaHCO_3$/kg did not result in a change in subsequent diet selection as expected. It was previously suggested that the degree to which the animal alters subsequent diet selection lies along a continuum so that even small changes in the internal environment would lead to small changes in subsequent diet selection (Provenza, 1995). However, the fact that adding $NaHCO_3$ to H did not result in a decrease in the proportion of food L selected during the subsequent diet selected, compared to sheep offered H unsupplemented, could be seen to disagree with this suggestion. It is unlikely that ruminants will try to keep their rumen

environment at a constant state where small deviations in the rumen environment warrant modifications in the diet selected to correct the deviations. More likely there will be a range of conditions that the animal will tolerate (e.g. Cooper *et al.*, 1995; Kyriazakis & Oldham, 1997). Should changes to the rumen environment exceed these 'tolerance' limits, modification to the diet selected could be expected. Therefore, larger, rather than smaller changes in the rumen environment should be expected to alter subsequent diet selection (Kyriazakis *et al.*, 1999). The fact that subsequent diet selection was not significantly altered when food H supplemented with $NaHCO_3$ was offered as a treatment food compared to H unsupplemented, yet offering food L, an even less disruptive food, significantly altered subsequent diet selection, supports this hypothesis.

An animal, given free and continuous access to a single food, over a period of time, can regulate its energy intake only by eating more or less of the food offered (Kyriazakis & Oldham, 1993). During Test B, food H was shown to be disruptive and as the sheep were unable to alter their diet, as no other food was offered, food intake and consequently daily ME intake were decreased. However, during Test C, a choice of foods was subsequently offered and sheep were able to modify their subsequent diet selection and achieve the same daily ME intake regardless of the treatment food that they were offered previously. The progressive increase and decrease in the proportion of food H selected with time, over the choice period, by sheep initially offered H or L respectively, is in support of this.

In conclusion, the sheep in this experiment appear to select a diet in agreement with the hypothesis that ruminants are faced with making a trade-off between the benefits of increasing energy intake against the costs of disrupting the rumen environment. The diet selected would be expected to firstly prevent further disruption to the rumen environment, which could be detrimental due for example to acidosis, and secondly to return the conditions in the rumen to within the accepted range as soon as possible (Kyriazakis, 1997). The results from this experiment are also in agreement with the idea that changes within the rumen of 'large' rather than 'small' magnitude due to the

consumption of a disruptive food, will alter subsequent diet selection by sheep (Kyriazakis & Day, 1998; Kyriazakis *et al.*, 1999). Ruminants make short-term adjustments in their diet selection in response to large changes in their current internal state and these enable them to meet their energy and nutrient requirements in the longer time scale, such as a day.

Chapter 6

General Discussion

The important thing is not to stop questioning.
Albert Einstein (1879-1956)

6.1 Introduction

The main objective of this thesis was to address the basis of the diet selection of sheep, with the aim of shedding some light on the rules that ruminants might use when selecting a diet. This was done by investigating the effects of offering foods with rapidly degradable nitrogen and rapidly fermentable carbohydrates, within the rumen, on the food intake and diet selection of sheep.

The micro-organisms in the rumen are highly proteolytic, and thus ensure that most of the protein entering the rumen from the foods consumed is degraded. The degraded protein is subsequently utilised by the micro-organisms to synthesise microbial protein (Van Soest, 1994). In many instances the majority of the protein that is absorbed from the digestive tract of the ruminant is therefore of microbial origin (Nolan and Leng, 1972; Dijkstra *et al.*, 1998). In view of the importance of microbial protein in the supply of protein to the ruminant host, it was hypothesised that growing sheep might select a diet to obtain an estimated 'optimal' effective rumen degradable protein (eRDP) to fermentable metabolisable energy (fME) ratio of 10:1 (Agricultural and Food Research Council (AFRC), 1993) from two foods that differ mainly in their eRDP content (Experiment 1, Chapter 2). The 'optimal' eRDP: fME ratio would be expected to maximise microbial protein synthesis, and minimise the energy costs associated with the detoxification of excess ammonia, absorbed from the digestive tract (Lobley *et al.*, 1995). As described in Chapter 2, the foods offered were presented to the sheep in a meal form to prevent chemical changes to the food ingredients that may occur during the pelleting process (Van Soest, 1982). However, as the food ingredients separated during the course of the experiment, demonstrated by a change in the chemical composition of the foods (Table 2.4), the outcome of the experiment was unclear.

The above hypothesis was therefore re-asserted in Experiments 2 and 3 reported in Chapter 3. However, the results did not support the hypothesis that sheep would avoid an excess consumption of rumen degradable protein (RDP) when given a choice. In fact

they chose foods with the greater contents of RDP. It was therefore questioned whether eRDP was a relevant dimension for the diet selection of ruminants. It was suggested that urea, which was used to alter the eRDP content of the foods offered, may have had other properties, such as a high buffering potential, that the sheep were selecting for when choosing a diet.

The hypothesis tested in Experiment 4 (Chapter 4) was that the expected preference of sheep for a food with adequate eRDP, supplemented with urea, would be reduced both by the addition of a buffer (sodium bicarbonate ($NaHCO_3$)) or by offering *ad libitum* access to hay. In both cases urea may no longer be needed as a buffer and the preference for the urea-supplemented food would then be expected to be reduced or even disappear. In Experiment 4, insufficient amounts of hay were consumed to reduce the preference for the urea-supplemented food. However, supplementing both foods with $NaHCO_3$ significantly reduced the preference for the urea-supplemented foods. It was proposed that the effect of $NaHCO_3$ addition on the diet selection of sheep was consistent with its buffering properties. When $NaHCO_3$ was added to both foods offered as a choice, the presumed need for urea to be used as a buffer was reduced, with a consequent decrease in the proportion of the diet selected as the urea-supplemented food. Effects of diet on buffering may override other diet selection objectives, such as the avoidance of an excess intake of RDP. This has led us to the idea of trade-offs in the diet selection of ruminants (Kyriazakis *et al.*, 1999). The sheep of Chapters 3 and 4 could be seen as facing a trade-off between the benefits of maintaining their rumen environment within a desired physiological range, against the costs of over-consuming RDP. The line of thinking that the sheep gave greater weighting to the benefits of maintaining their rumen within a desired physiological range led into the second line of research (Chapter 5). Chapter 5 investigated the hypothesis that one of the objectives of the diet selection of ruminants is to maintain the rumen environment within a certain physiological range.

Experiment 5 (Chapter 5) studied the effects of short-term consumption of foods that differ in energy density (ED) and /or $NaHCO_3$ supplementation, hence differing in their

potential effects on the rumen environment, on subsequent food intake and diet selection of sheep. The low ED food was expected to reduce the ability of sheep to meet their energy requirements, due to its bulkiness. It was hypothesised that the proportion of the low ED food selected in the diet, when sheep are offered a choice between a high and low ED food, would dependent upon the food previously consumed and hence the disturbance caused to the rumen environment. That is, animals that have consumed a high ED food will select a diet in favour of the low ED food and vice versa. The results showed that short-term consumption of foods that differ in ED, and hence in their potential impact on the rumen environment, significantly affect subsequent diet selection (Test C, Chapter 5). This was in agreement with the hypothesis that ruminants select a diet to help maintain the rumen environment within a certain physiological range (Cooper *et al.*, 1995, 1996; Faverdin, 1999; Kyriazakis *et al.*, 1999). These results are again consistent with the idea of trade-offs in the diet selection of ruminants. Sheep could be seen as facing a trade-off in their diet selection when selecting a diet to meet their energy requirements, and yet maintaining their rumen environment within a desired physiological range.

The principal issues raised by the experimental studies of this thesis, which warrant further discussion in this chapter, are:

- What are the possible mechanisms that monitor and control the consumption of RDP and urea?
- Are there any other explanations as to why sheep selected for the urea-supplemented foods other than that urea has properties, such as a high buffering potential, that the sheep were selecting for when choosing a diet?
- If other explanations can account for sheep selecting for the urea-supplemented food, can these be consistent with the decrease in this preference for the urea-supplemented food by $NaHCO_3$ supplementation?

This final chapter deals first with the principle issues described above. The chapter then goes on to consider the idea of trade-offs in the in diet selection of ruminants, together with some suggestions for future research.

6.2 Effects of adding urea to foods

A lot of work over the years has looked at the effects of adding urea to nitrogen deficient foods. Various authors have shown that supplementing a food deficient in nitrogen, with urea, increases digestibility (Carro and Miller, 1999), food intake (Ørskov *et al.*, 1971; Egan and Doyle, 1985; Ternouth *et al.*, 1993; Manyuchi *et al.*, 1994) and live weight gain (Ørskov *et al.*, 1972; Kyriazakis and Oldham, 1993). The results from Experiments 2 and 3 (Chapter 3) are in agreement with these.

Ammonia, resulting from protein degradation in the rumen, in excess of the capacity of the micro-organisms to capture and utilise it, could be associated with energy costs for its detoxification once it has left the digestive tract (Lobley *et al.*, 1995; Parker *et al.*, 1995). High ammonia concentrations, yielded from foods consumed, can also be detrimental to the ruminant by causing ammonia toxicity (Chalupa, 1968; Visek, 1968). Therefore it would be advantageous for the ruminant to avoid an excess consumption of ammonia generating foods, and hence excess RDP. However, sheep offered a single food calculated to contain, at least, adequate amounts of eRDP, supplemented with urea, did not reduce their food intake, and hence did not avoid an excess consumption of RDP (Milton *et al.*, 1997; Shain *et al.*, 1998). It was suggested in Chapters 3 and 4 that the sheep may have coped with the additional RDP from urea, and hence the expected increase in rumen ammonia concentrations, by changing their feeding behaviour (Loosli and Warner, 1958). More frequent, and smaller, meals may allow the extent of the rise in ammonia concentration to be reduced (Dulphy *et al.*, 1992; Kahn, 1996). By altering their feeding behaviour, sheep could continue to meet their requirements for other nutrients and energy.

Emmans (1991) suggested that the results obtained from single food treatments can be helpful in interpreting the diet selection made by animals offered food choices (e.g. Kenney and Black, 1984; Kyriazakis and Oldham, 1993; Cooper *et al.*, 1995, 1996). However, the rules that govern the feeding behaviour of sheep offered a choice will need

to be richer than those used to determine the intake of a single food (Arsenos and Kyriazakis, 2001). For example, sheep may be prepared to consume significant amounts of a food when offered on its own, yet avoid it when offered together with another food as a choice. This would be consistent with observations made on other animals (Kyriazakis and Emmans, 1991).

On the one hand it could be expected that when sheep are offered a choice between an eRDP deficient food and the same food supplemented with urea, the supplemented food would be predominantly selected (Kyriazakis and Oldham, 1993). This is because the urea-supplemented food has been shown to be beneficial in terms of food intake and weight gain when offered alone on an *ad libitum* basis. The sheep of Experiments 2 and 3 (Chapter 3) showed a preference for the urea-supplemented food when such a choice was offered. On the other hand the addition of urea to a food already containing adequate eRDP was not shown to be beneficial in terms of food intake or live weight gain (Chapters 3 and 4). Therefore, it could be expected that sheep would avoid such a food when offered a choice and therefore minimise excess RDP consumption (Tolkamp *et al.*, 1998b). However, sheep of Chapters 3 and 4 did not avoid an excess consumption of RDP, in fact they chose the food with the greater RDP content.

Having completed the experiments reported in this thesis, the basis of protein selection by ruminants still remains unclear and raised questions on what are the possible mechanisms that monitor and control the consumption of RDP and urea. The following sections will discuss the possible mechanisms that monitor and control the consumption of RDP. Subsequently hypotheses based on the results from Experiments 2 and 3 (Chapter 3) to explain why sheep selected for the urea-supplemented food will be described. In Section 6.3, these hypotheses will again be examined to determine if they are consistent with the decrease in preference for the urea-supplemented food by $NaHCO_3$ supplementation (Experiment 4)

6.2.1 *Possible mechanisms that monitor and control the consumption of RDP*

Tolkamp *et al.* (1998b) showed that cows were prepared to over-consume metabolisable protein (MP) in order to meet their eRDP requirements when offered a choice between a high and a low protein food. When urea was added to both the high and low protein foods, the cows reduced the proportion of the high protein food in their diet. These results were viewed as evidence of behaviour that ruminants aim to regulate their eRDP intake and avoid both deficient and excessive amounts of eRDP. This suggests that ruminants monitor and control the consumption of RDP *per se*.

Rumen degradable food proteins are hydrolysed to peptides, amino acids and ammonia by rumen micro-organisms. However, the monitoring of peptides and amino acids *per se* as a means of monitoring and controlling the consumption of RDP would not explain the food intake and diet selection responses of ruminants to sources of non-protein nitrogen (NPN) such as urea (Kyriazakis and Oldham, 1993; Tolkamp *et al.*, 1998b). Peptides and amino acids not utilised by the micro-organisms may be hydrolysed to ammonia (Van Soest, 1994). In addition urea and other NPN sources are rapidly hydrolysed to ammonia (Wallace and Cotta, 1988).

Ammonia not utilised by the micro-organisms within the rumen can be absorbed from the rumen and other parts of the gastro-intestinal tract such as the omasum, lower part of the small intestine and cecum (Meissner *et al.*, 1993). The ammonia absorbed from the digestive tract is carried, in the blood, to the liver where it is detoxified to urea (Lobley *et al.*, 2000). High concentrations of ammonia leaving the digestive tract can result in the capacity of the liver to convert ammonia to urea being exceeded (Chalmers *et al*, 1971). This can subsequently result in the concentration of ammonia in the peripheral blood increasing, and hence ammonia toxicity (Visek, 1968; McEvoy *et al.*, 1997; Ortolani *et al.*, 2000). Therefore, there is a strong argument for the concentration of ammonia to be monitored as an indication of the amount of RDP consumed. Monitoring the concentration of ammonia, generated from the foods consumed, should occur either

within the liver or within organs where the ammonia is present, prior to its arrival at the liver. This would allow rapid detection of high ammonia concentrations, generated from the food consumed, and would allow the animal to limit further consumption of this food prior to ammonia concentrations in the peripheral blood increasing.

The rumen is ideally situated to detect concentrations of ammonia generated from the foods consumed, as it is here that the majority of the ammonia is liberated (Van Soest, 1994). Detection of ammonia concentrations within the rumen would allow a rapid response in the feeding behaviour of the ruminant. This rapid response would allow the ruminant to avoid further consumption of the high ammonia generating food (Tolkamp *et al.*, 1998b), hence avoid an excess consumption of RDP, or to increase the amount of ammonia generating foods, hence avoid RDP deficiency. However, the ammonia concentration within the rumen is a poor indicator of whether deficient or excess amounts of RDP have been consumed. When the diet is nitrogen deficient, urea synthesised from the liver may be recycled back to the rumen (Brun-Bellut, 1996). The recycled urea would then be hydrolysed to yield ammonia, increasing the ammonia concentration within the rumen (Van Soest, 1994). Therefore the ammonia concentration in this circumstance would not be a true representation of the RDP consumed. In addition, high ammonia concentrations within the rumen do not necessarily correspond with the concentration of RDP consumed in excess of requirements, as some of the ammonia generated from dietary protein and NPN hydrolysis will subsequently be utilised by the micro-organisms to synthesise microbial protein.

As ammonia absorbed from the digestive tract is filtered through the liver by the hepatic portal vein (Frandson and Spurgeon, 1992), the hepatic portal vein and the liver are well placed to play a role in monitoring and controlling the intake of ammonia generating foods and hence RDP. Ruminants may monitor the amount of RDP consumed by the concentration of ammonia that arrives at the liver. Therefore, when low concentrations of ammonia arrive at the liver, as there will always be some ammonia that leaves the digestive tract (Nolan, 1975), the ruminant may perceive the diet consumed as RDP

deficient. When high concentrations of ammonia arrive at the liver, the ruminant may perceive the diet consumed as containing excess RDP.

When applying this idea to the foods offered in Chapter 3, it could be expected that adding urea to a food containing adequate eRDP (food H) would increase the concentration of ammonia arriving at the liver and therefore result in this food being perceived as containing excess RDP. However, sheep still selected for such a food when offered a choice. The sheep even selected for the urea-supplemented food when the food to which the urea was added was calculated to contained excess RDP (food P). To formulate food P, a proportion of the wheat in food H was substituted with soya bean meal. From tables on protein degradation rates, the protein in soya bean meal is degraded a lot slower than that of wheat (Ministry of Agriculture, Fisheries and Food (MAFF), 1990). Combining the effects of degradation rate and protein content, food P may yield a similar concentration of ammonia as food H per unit time. Therefore, the concentration of ammonia arriving at the liver subsequent to the consumption of either food H or food P may be similar. This may therefore result in similar proportions of the urea-supplemented foods being selected by sheep irrespective of whether the urea was added to the H or P food. However, this does not explain why sheep offered a food that contained, at least, adequate eRDP with respect to the fME content of the food, and this food supplemented urea, selected for the urea-supplemented food.

One possible explanation is that other factors may have decreased the net absorption of ammonia from the digestive tract. The sheep may have selected for the urea-supplemented food as they perceived this diet as an eRDP sufficient one and not realise that an excess RDP is actually being consumed due to the reduced ammonia concentration arriving at the liver. Alternatively, RDP intake may not be limited unless the amount consumed generates toxic concentrations of ammonia. The fact that sheep selected for the urea-supplemented food in Experiments 2, 3 and 4 may therefore be due to ruminants having evolved to maximise RDP consumption unless the foods consumed generate toxic concentrations of ammonia. Finally, eRDP may not be the relevant

dimension in the diet selection of ruminants. Urea, which was used to alter the eRDP content of the foods offered to sheep, may have had other properties, such as a high buffering potential, that the sheep were selecting for when choosing a diet. These suggestions are developed in the following sections with reference to the results presented in Chapter 3.

6.2.1.1 *Ammonia absorption from the digestive tract was reduced*

It has previously been shown that urease activity is pH sensitive and that the rate of urease activity is reduced with a fall in pH (Cabrera *et al.*, 1991; Fahmy *et al.*, 1998). The foods offered in the Chapter 3 were all high energy, readily fermentable, concentrate foods. These foods are often associated with a decline in rumen pH (Counette *et al.*, 1979; Forbes and Barrio, 1992). This fall in rumen pH could therefore potentially result in a decrease in the rate of urea hydrolysis through a decrease in the rate of urease activity (Cabrerea *et al.*, 1991; Fahmy *et al.*, 1998). In turn this would reduce the ammonia concentration within the rumen (Lana *et al.*, 1998) and therefore the ammonia available for absorption.

In the previous section, it was suggested that the RDP content of the diet consumed might be monitored, and subsequently controlled, by the concentration of ammonia that arrives at the liver. Factors, such as a low rumen pH, may decrease the concentration of ammonia leaving the digestive tract and arriving at the liver (Lana *et al.*, 1998). Therefore, what we considered to be an eRDP sufficient diet for a growing sheep, may be perceived by the sheep as an eRDP deficient diet due to the reduced concentration of ammonia arriving at the liver. The sheep of Experiments 2 and 3 may have positively selected for urea to increase their RDP intake. The increased amount of urea consumed may have subsequently resulted in an increase in the concentration of ammonia arriving at the liver. This would be achieved through increasing the urea substrate available for hydrolysis, given that the enzyme, urease, was in excess to the substrate, urea (Russell *et al.*, 1992). Therefore, the sheep offered a choice between an eRDP adequate food and

the same food supplemented with urea may therefore perceive the diet containing a large proportion of the urea-supplemented food as an eRDP adequate, yet not excessive one.

If the unsupplemented food was considered to be deficient in eRDP by the sheep, it could be expected that when offered alone, sheep would increase their intake of such a food to obtain what they perceive as 'sufficient' eRDP. Such a compensatory increase in food intake is common in simple-stomached animals (Musten *et al.*, 1974; Kyriazakis *et al.*, 1990, 1991). However, such a compensatory increase in food intake is often absent in ruminants (Chapter 3; Kyriazakis and Oldham, 1993; Ternouth *et al.*, 1993). In the General Introduction it was suggested that the absence of a compensatory increase in food intake might be due to the fact that increasing the intake of a readily fermentable concentrate would result in an increase in the VFA concentration within the rumen. Due to the negative effects of a high VFA concentration on rumen pH and in turn on food intake, as described in Section 1.5 of the General Introduction, this would be counter-productive to the animals attempting to increase RDP consumption. Therefore, the sheep offered a single food, which they perceive as RDP deficient, may not have increased their food intake to obtain what they perceive as sufficient eRDP, due to the negative effects of the rapidly fermentable carbohydrates present in the concentrate foods.

The hypothesis suggests that ruminants will select a diet that they perceive as an eRDP adequate, yet not excessive one, through monitoring the concentration of ammonia arriving at the liver. The sheep of Experiments 2 and 3 included a large proportion of the urea-supplemented food in their diet, as they perceived this diet to meet their eRDP requirements, yet not contain excess RDP. This was due to a reduced concentration of ammonia arriving at the liver as a result of a low rumen pH. This hypothesis can explain why sheep of Experiments 2 and 3 (Chapter 3) selected for the urea-supplemented foods.

6.2.1.2 *Ruminants do not recognise excess RDP unless it is toxic*

In Section 6.2.1, an alternative hypothesis to the one described above was that ruminants might have evolved to maximise RDP consumption unless the ammonia generated

reaches toxic concentrations rather than maintaining eRDP consumption at an optimum concentration (Section 6.2.1.1). Therefore the sheep would not perceive the diet containing 'adequate' eRDP as deficient, but would still show a preference for the urea-supplemented food as this food contains a greater RDP content.

It is well recognised that ruminants are especially well adapted to survive and even thrive in environmental conditions that are unfavourable to other large mammals. One of these adaptations is the ability of the ruminant to conserve body nitrogen and to use sources of nitrogen, such as NPN, unavailable to other mammals (Leng and Nolan, 1984). Taking this into account, ruminant 'ancestors' may have evolved preferences for foods with higher RDP contents, especially when the RDP content of many of the foods available could be expected to be low. Such a preference may have developed because the maintenance of the rumen eco-system is crucial to the metabolism of the host (Theodorou and France, 1993). Also, it has been suggested that the foods which generate ammonia in the rumen signal that essential amino acids will follow (Faverdin, 1999). In circumstance where foods containing excess RDP are rare, if at all encountered, mechanisms that limit the consumption of ammonia generating foods, and hence RDP, may not exist. Therefore ruminants may select for foods that contain greater concentrations of RDP unless the amount of ammonia generated becomes toxic.

One situation where this hypothesis does not predict the diet selected by the sheep of Experiments 2 and 3 is when these sheep were offered a choice between a food that contained 'at least' adequate eRDP and the same food supplemented with 12.5g urea/kg. The preference for the food with the higher urea content tended to be stronger when the supplemented food had 25g rather than 12.5g urea/kg. However, the addition of only 12.5g urea/kg may not have been enough for any difference in the RDP content to be detected. With no difference detected the sheep would be expected not to form a preference for the urea-supplemented food resulting in a diet selection that was not significantly different from random (0.5), as was the case. Sheep offered a choice between the eRDP adequate food (food H) and this food supplemented with 25g urea/kg,

or a food containing excess RDP (food P) and this food supplemented with 25g urea/kg (Experiments 2 and 3, Chapter 3), all showed a preference for the urea supplemented food. That is, all sheep selected the food with the greater RDP content, in agreement with this hypothesis. No difference in the proportion of the urea-supplemented food selected would be expected between groups offered either the H or the P foods as the ammonia generated per unit time by food H and P may be similar as described above.

This hypothesis suggests that ruminants will select a diet to maximise RDP intake rate when offered a choice unless the ammonia generated is toxic. This hypothesis can account for why sheep of Experiments 2 and 3 (Chapter 3) selected for the urea-supplemented foods.

6.2.1.3 *Urea has some beneficial property other than providing nitrogen*

There were no apparent associated costs with consuming excess RDP as shown by the weight gain of sheep offered a single food supplemented with urea. However, there were also no apparent benefits of supplementing eRDP adequate foods with urea in terms of food intake and weigh gain. It has previously been suggested that for an animal to form a preference for a food, the food must have some beneficial property (Provenza, 1995). This led to the line of thinking that urea had some beneficial property other than providing nitrogen, such as a high buffering potential, that the sheep may have been selecting for when choosing a diet.

The foods used in the experiments reported in Chapters 3 and 4 were low in fibre, of high fermentability and were pelleted. On such foods a reduction in rumination (Campion and Leek, 1997) and a lower pH in the rumen would be expected (Chapter 5). This lower rumen pH could reduce microbial activity (Russell *et al.*, 1979) and could be detrimental to the host (Owens *et al.*, 1998). It has been suggested that one of the objectives of ruminants when selecting a diet is to maintain the rumen environment within a certain physiological range (Parsons *et al.*, 1994; Cooper *et al.*, 1995; Kyriazakis *et al.*, 1999). It is possible that the diets selected by the sheep were, at least in

part, a means of reducing the potential disturbance of the rumen environment and had nothing to do with protein either as eRDP or as MP.

Haaland *et al.* (1982) found that the pH and buffering capacity of the rumen fluid was increased significantly when the crude protein content of the food was increased from 11% to 17%. The hydrolysis of proteins can result in the production of ionizable α–amino groups and subsequently ammonia, both of which can reduce the amount of hydrogen ions within the rumen fluid and hence act as buffers and increase rumen pH (Stryer, 1988). Urea may also act in a similar way through the mild buffering effects of the ammonia yielded when hydrolysed, as suggested by Poos *et al.*, (1979). Therefore, sheep may have selected for the foods supplemented with urea in an attempt to buffer the rumen environment and maintain a higher pH on the high quality foods used (Phy and Provenza, 1998a). Therefore it was suggested that the sheep were prepared to over consume RDP in an attempt to maintain the rumen environment within a desired physiological range. This would help explain why sheep offered a food already containing excess RDP, with respect to the fME content of the food, and the same food supplemented with urea, selected the urea supplemented food.

This hypothesis suggests that ruminants selected the urea-supplemented foods due to the buffering potential of these foods. If this is the case, it could be expected that the proportion of the urea-supplemented food in the selected diet would be reduced when $NaHCO_3$ was supplemented to both foods offered as a choice as urea would no longer be needed as a buffer. This theory was tested in Experiment 4, Chapter 4.

6.3 Sodium bicarbonate modifies the diet selection for the urea-supplemented food.

Having completed the experiments reported in Chapter 3, the issue of the basis of diet selection for protein still remained unclear due to the sheep not avoiding an excess

consumption of RDP. The previous section considered a number of hypotheses to try and explain why the sheep selected for the urea-supplemented food. These hypotheses could all account for sheep selecting for the urea-supplemented food. The question therefore is, can they be consistent with the decrease in this preference for the urea-supplemented food by $NaHCO_3$ supplementation?

The intermediate variable in the possible explanation why sheep selected the urea-supplemented food approached in Chapter 4 (described in section 6.2.1.3) was rumen pH. In an ideal situation rumen pH should therefore have been measured. However, rumen pH was not measured due to reasons that were both technical and ethical. Our past experience led us to decide to have 7 replicate animals on each of the 14 treatments (Chapter 4). With a total of 98 animals it was impossible to measure the rumen pH of all of them. The options were to sub-sample or to have yet more animals on the treatments for the sole purpose of measuring rumen pH. However, the interpretation of data from spot samples of rumen fluid from animals fed *ad libitum* is not straightforward unless so many are taken through a 24-hour period that the welfare of the animals is prejudiced. The option of continuous monitoring of rumen pH using electrodes planted in the rumen was not available and even if such a technique were available the positioning of the probe would have led to further problems (McBride, personal communication). As it was presumed that sheep were selecting for the food with the greater buffering potential, the buffering capacity of each food offered was measured as an indication of the effect of these foods on the rumen environment.

6.3.1 *Sodium bicarbonate increases the absorption of ammonia from the digestive tract*

In Section 6.2.1, it was suggested that the RDP content of the diet consumed might be monitored, and subsequently controlled, by the concentration of ammonia that arrives at the liver. If factors decrease the concentration of ammonia received by the liver, sheep

may positively select for urea to obtain what they perceive as an eRDP sufficient, but not excessive, diet (Section 6.2.1.1).

$NaHCO_3$ has previously been shown to be an effective dietary buffer for ruminants when added to foods of low fibre and high energy content (Ha *et al.*, 1983; Hart and Polan, 1984). Therefore it was expected that $NaHCO_3$ supplementation would have beneficial effects on the rumen environment by reducing the decline in pH when the high ED, low fibre food was consumed (Chapter 5).

As a result of an increase in rumen pH, due to the consumption of $NaHCO_3$, urease activity and hence ammonia yielded from the consumed urea may also increase (Cabrera *et al.*, 1991; Fahmy *et al.*, 1998; Lana *et al.*, 1998). An increase in the ammonia yield would result in an increase in the concentration of ammonia reaching the liver. As the concentration of ammonia arriving at the liver increases, the sheep may then perceive the diet consumed as containing excess RDP and subsequently decrease the proportion of the urea-supplemented food in their diet (Tolkamp *et al.*, 1998b). This was observed in Experiment 4 (Chapter 4). The idea that, sheep may positively select for urea to obtain what they perceive as an eRDP sufficient, but not excessive, diet due to a reduction in the ammonia arriving at the liver, therefore cannot be rejected on the basis of the diet selected by the sheep in Experiment 4.

This hypothesis assumes that the enzyme, urease, is in excess to the substrate, urea and that consumption of RDP is monitored and controlled only by the concentration of ammonia arriving at the liver. To differentiate this hypothesis from the one described in section 6.2.1.3, further research would be required to determine if the enzyme, urease, was in fact in excess of the substrate, urea. Also the mechanisms that monitor and control the consumption of RDP need to be defined. However, from the results presented in this thesis, this hypothesis cannot, at present, be rejected and may help explain the protein selection of ruminants.

6.3.2 *The concentration of RDP consumed generated toxic ammonia concentrations when $NaHCO_3$ was added.*

In Section 6.2.1.2 it was hypothesised that ruminants may select for foods containing the greater concentrations of RDP unless the amount of ammonia generated reached toxic levels. Therefore the supplementation of $NaHCO_3$ to both foods offered as a choice would not be expected to alter the diet selected, as $NaHCO_3$ does not alter the RDP content of the food. However, supplementing both foods with $NaHCO_3$ significantly reduced the preference for the urea-supplemented food.

If the amount of ammonia leaving the digestive tract was reduced as suggested in Section 6.2.1.1, then an increased amount of urea could be consumed without ammonia toxicity occurring. Increasing the amount of ammonia leaving the digestive tract through $NaHCO_3$ supplementation (Cabrera *et al.*, 1991; Fahmy *et al.*, 1998) may have subsequently resulted in the capacity of the liver to convert the ammonia to urea being exceeded (Chalmers *et al.*, 1971). Therefore the sheep would be expected to reduce the proportion of urea-supplemented food in their diet to avoid ammonia toxicity. However, sheep offered the single, excess RDP food, supplemented with both urea and $NaHCO_3$ did not display any signs of ammonia toxicity (Chalupa, 1968) nor did they reduce their food intake to reduce the consumption of the high ammonia generating food (Wilson *et al.*, 1975. Therefore this hypothesis cannot account for why sheep selected for the urea-supplemented food, as it is not consistent with the decrease in preference for the urea-supplemented food by $NaHCO_3$ supplementation.

6.3.3 *Sodium bicarbonate reduces the need for urea as a buffer*

It was suggested in Chapter 3 and developed in Chapter 4 that sheep were possibly selecting diets high in urea, which has been shown to elevate rumen pH (Kyriazakis and Oldham, 1997; Archimède *et al.*, 1999), as a means of decreasing the potential

disturbance of the rumen environment created by the consumption of the readily fermentable concentrate food to which the urea was added (Phy and Provenza, 1998 a, b). In circumstances where rumen pH is not lower than that desired for maximum rumen function (Erdman, 1988), excess RDP from urea has been suggested to have a negative effect on the efficiency of nutrient utilisation. For example, the conversion of ammonia back to urea within the liver costs the animal about 50 MJ/kg N (Tyrrell *et al.*, 1970). Excess RDP may also affect the energy and nitrogen metabolism of the animal (Lobley *et al.*, 1995). Therefore the over-consumption of urea, and hence RDP, has associated costs other than the risk of ammonia toxicity. If urea is no longer needed as a buffer, due to an alternative buffer being supplied, then it was expected that the preference for the urea-supplemented food would be reduced or may even disappear. A supplement of $NaHCO_3$ to both foods offered as a choice, and hence its consumption made unavoidable, significantly decrease the preference for the urea-supplemented food. The food supplemented with urea was not totally avoided as the urea had a greater buffering capacity than the $NaHCO_3$ (Table 4.2). A movement towards the avoidance of an excess consumption of RDP when $NaHCO_3$ was added to both foods offered as a choice was in agreement with the results of Tolkamp *et al.* (1998b). In that experiment, there was a decrease in the preference for the food with the higher RDP content when urea was added to two forage based foods offered as a choice. Therefore, providing that the source of RDP does not have other properties that are relevant in the test situation, the sheep of Chapter 4 and the cows in Tolkamp *et al.*'s (1998b) experiment, appear to follow the hypothesis that ruminants avoid an excess consumption of RDP (Kyriazakis and Oldham, 1993).

The fact that $NaHCO_3$ supplementation reduced the preference for the urea supplemented foods suggests that sheep of Chapters 3 and 4 may have selected foods for their effects on the rumen environment, such as their buffering capacity (Parsons *et al.*, 1994; Cooper *et al.*, 1995; Kyriazakis *et al.*, 1999; Chapter 5). Therefore, the sheep of Chapters 3 and 4 can be seen as facing a trade-off between the benefits of selecting

foods with a high buffering capacity, such as maintaining their rumen environment within a desired physiological range, against the costs of over consuming RDP.

6.4 The idea of trade-offs in diet selection

Food choices lead to both benefits, such as meeting nutrient requirements, and costs, such as those arising from the consumption of toxins (Belovsky and Schmitz, 1994). In the simple situation where a combination of the foods offered as a choice leads to the situation where benefits outweigh costs, animals are expected to select a diet that will meet their nutrient requirements (Emmans, 1991). Another choice may have both higher benefits and higher costs and the animal would then have to make a trade-off between the increased benefits and costs in order to come to a decision in relation to its diet selection. An animal can be assumed to make the choice that maximises the difference between the benefits and costs (Kyriazakis *et al.*, 1999).

Previously, the idea of trade-offs in the diet selection of ruminants has been used to account for the selection of diets from foods that differed in their nutrient and toxin concentrations (Belovsky and Schmitz, 1994). The animal was considered to make a trade-off between increasing the benefits (nutrient intake) and minimising the costs (toxin intake). In the previous section, another example of a trade-off was described. It was suggested that one of the diet selection objectives of ruminants is to maintain the rumen environment within a desired physiological range (Cooper *et al.*, 1995, 1996; Faverdin, 1999; Kyriazakis *et al.*, 1999). Therefore, when offered a choice of two foods, both of which were readily fermentable concentrates, the sheep selected the food with the greater buffering potential (Chapters 3 and 4). However, this resulted in an excess consumption of RDP, as the food with the greater buffering potential was the food supplemented with urea. Little weighting appeared to be given to the consumption of excess RDP and its associated costs (Lobley *et al.*, 1995). However, the urea-supplemented food was not predominantly selected (Chapters 3 and 4). Increasing RDP

consumption further by increasing the proportion of the urea-supplemented food in the diet would have increased the associated costs. A point may then be reached where the cost of consuming the urea-supplemented food exceeded the associated benefits, namely the buffering potential of the food. Therefore, the sheep selected a significant, but not predominant, proportion of the urea-supplemented food as a result of maximising the difference between the benefits and costs (Kyriazakis *et al.*, 1999). Supplementing both foods offered as a choice with $NaHCO_3$ (Chapter 4) reduced the suggested need for urea as a buffer. The foods containing adequate or excess eRDP, supplemented with $NaHCO_3$ and urea were expected to be associated with greater costs than the foods supplemented with $NaHCO_3$ but not urea. Therefore, as sheep are predicted to maximise the difference between the benefits and costs, the proportion of the urea-supplemented food in the selected diet, in the presence of $NaHCO_3$, was reduced.

The sheep of Chapters 3 and 4 can therefore be seen as facing a trade-off between the benefits of selecting foods with a high buffering capacity, such as maintaining their rumen environment within a desired physiological range, against the costs of over consuming RDP. The line of thinking that the sheep gave greater weighting to the benefits of maintaining their rumen within a desired physiological range led on to the second line of research (Chapter 5). Chapter 5 investigated the hypothesis that one of the objectives of the diet selection of ruminants is to maintain the rumen environment within a certain physiological range.

The results from Experiment 5 showed that, when the rumen environment was challenged by the consumption of a high energy, readily fermentable, therefore disruptive food, the initial response of the sheep was to alter their diet selection. Sheep increased the proportion of the food that had the greater potential to buffer the rumen environment in agreement with the results reported in Chapter 3 and 4. This agreed with the hypothesis that modification in the diet selected would be firstly to prevent further disruption to the rumen environment which could be detrimental e.g. acidosis, and

secondly to return the conditions in the rumen to within the accepted range as soon as possible (Kyriazakis, 1997).

However, the incorporation of a lower ED food into the diet can result in an inability of the animal to meet its energy requirements due to its bulkiness. This was shown in Test A (Experiment 5, Chapter5). In this test, the time that food was offered was restricted to 8 hours daily. This resulted in a significantly lower daily metabolisable energy (ME) intake and consequently live weight gain daily compared to sheep offered the same choice of foods for 24 hours daily (Newman *et al.*, 1994; Parsons *et al.*, 1994). Had the sheep offered food for the restricted time period selected a greater proportion of the high ED food, yet maintained an intake similar to what was actually achieved, ME intake and potentially live weight gain, would have been similar between the two groups. However, increasing the proportion of the high ED food, in conjunction with an increased food intake could potentially result in an increased yield of VFA's per unit time (Baik *et al.*, 1997) and a fall in rumen pH. This would be counter productive to the animal attempting to increase its ME intake (Phy & Provenza, 1998a). Therefore, the diet selected again leads us to the idea of trade-offs in the diet selection of ruminants. In the case described here, the trade-off arises from the benefits of selecting a higher ED food, hence meeting the energy requirements of the animal, against the cost of disrupting the rumen environment (Kyriazakis *et al.*, 1999).

The sheep of Chapters 3 and 4 may try *both* to maintain rumen pH above a certain value *and* to avoid an excess RDP intake. The sheep of Chapter 5 may try *both* to maintain rumen pH above a certain value *and* to meet their daily ME requirements. In all cases, where the two objectives conflicted, maintaining the rumen environment within a desired physiological range appeared to take priority (Cooper *et al.*, 1995). Maintaining the rumen environment within a certain physiological range appears to underline the basis of ruminant diet selection and makes evolutionary sense, as this would help sustain food intake and yields of energy and protein.

The idea of benefits and costs that are weighted in some way by the animal is helpful in considering the diet selection of ruminants. However, there then is a need to be able to quantify these weightings so that the cost and benefits of a particular diet and subsequently the diet selection can be predicted. As described in this section, it was suggested that sheep gave greater weighting to maintaining the rumen environment within a certain physiological range than avoiding an excess consumption of RDP or meeting daily ME requirements. Therefore these sheep selected the foods that had the greater buffering potential. The determination of rules, or objectives, used by ruminants when selecting a diet, such as maintaining the rumen conditions within a certain physiological range, are a necessary first step. They will help in the understanding and predicting of the diet selection of ruminants in more complex situations as theories of diet selection seek to be general.

6.5 Future directions

6.5.1 *Will sheep over-consume RDP, supplied by urea, when forage or an alternative buffer is offered?*

The objective of Experiment 4 (Chapter 4) was to test two hypotheses. H1: the expected proportion of a urea supplemented food in the selected diet would be reduced by offering hay *ad libitum* along with the choice of concentrate foods. H2: supplementing both foods offered, as a choice, with $NaHCO_3$ would reduce the expected proportion of a urea-supplemented food in the selected diet. In both cases urea may no longer be needed as a buffer to the same extent and preference for the urea-supplemented food would then be expected to be reduced or even disappear.

When $NaHCO_3$ was supplemented to both foods offered as a choice, the proportion of the urea-supplemented food in the selected diet did decrease in agreement with the hypothesis. However, only trivial amounts of hay were consumed. Therefore no change

in the diet selected was observed when hay was offered *ad libitum* along with a choice of foods. It was surprising that sheep did not consume a greater quantity of hay than they did as many authors have suggested that ruminants have an 'appetite' for long forage (e.g. Campion and Leek, 1997). Others (e.g. Engku Azahan and Forbes, 1992; Cooper *et al.*, 1996) have found that ruminants may voluntarily include a large proportion of long forage in their diet.

One possible explanation discussed in Chapter 4 was that the amount of concentrate consumed prior to, and during, the experiment maintained the pH of the rumen at a level too low for the hay to be digested (Mould and Ørskov, 1984; Calsamiglia *et al.*, 1999). Even though the hay was offered over four weeks, a period that should be sufficient for the cellulolytic bacteria to re-establish (Mould and Ørskov, 1984), the concentrates consumed may have continuously maintained the pH at a level too low for these bacteria to thrive (Russell and Hespell, 1981). Therefore, to determine if the lack of hay consumption was due to the concentrate feeding, hay should be offered *ad libitum* during the adaptation period and during the choice period, i.e. sheep should always have continuous access to hay. A continuous access to hay may prevent a fall in rumen pH caused by the consumption of concentrate foods. Should the experimental foods of Chapter 4 be offered again to sheep that have continuous access to hay, the diet selected would be expected to follow the predictions that sheep would select a diet that avoided both deficient and excessive amounts of RDP (Tolkamp *et al.*, 1998b).

A supplement of $NaHCO_3$ to both foods offered as a choice in Experiment 4 (Chapter 4), and hence its consumption made unavoidable, significantly decreased the proportion of the urea-supplemented foods in the selected diet. Once an alternative buffer was consumed the expected need for urea, as a buffer reduced and as a consequence sheep reduced the amount of excess RDP consumed. However, the food supplemented with urea was not totally avoided as the urea had a greater buffering capacity than the food supplemented with $NaHCO_3$ (Table 4.3). Therefore, it was expected that the consumption of the food supplemented with $NaHCO_3$ was not capable of increasing the

pH of the rumen to within the desired physiological range without some of the urea-supplemented food being consumed. It is possible that if the buffering capacity of the foods offered as a choice were even greater, the urea-supplemented food would be avoided to a greater extent. That is sheep could completely avoid an excess consumption of RDP, as no urea would be required to buffer the rumen environment. However, higher concentrations of $NaHCO_3$ could increase rumen osmolality that in turn, could result in a decrease in food intake as found by Carter and Grovum (1990) and Cooper *et al.* (1996) and reported on Test B (Experiment 5, Chapter5). Therefore, the experiment reported in Chapter 4 could be repeated with different buffers that have greater acid consumption capacities such as magnesium oxide and potassium carbonate (Erdman, 1988). It would therefore be possible to see if the preference for the urea-supplemented food could be altered to different degrees depending on the pH of the rumen environment and the buffering capacity of the foods to which urea was supplemented.

As mentioned in section 6.3, the intermediate variable in the possible explanation why sheep selected the urea-supplemented food was rumen pH. However the rumen pH was not measured in the experiments due to technical and ethical reasons. In an ideal situation, to strengthen the argument that urea increases rumen pH, and that ruminants select a diet to maintain the rumen environment within a certain physiological range, rumen pH measurements should be collected. With advances in technology, methods of measuring rumen pH accurately, without compromising the welfare of the animal, may become available. Such measurements would provide support that ruminants over-consumed RDP due to the buffering potential of the urea. However, proving that urea was selected for its buffering properties, does not provide information on the basis of protein selection by ruminants.

6.5.2 *What is the basis of protein selection by ruminants?*

Four experiments reported in this thesis (Chapters 2, 3 and 4) investigated whether the diets selected by ruminants would be those that met the eRDP requirements of the

animals and at the same time avoid excess consumption of RDP. This hypothesis was originally suggested by Tolkamp *et al.*, (1998b). However, when Tolkamp *et al.* tested this hypothesis the foods offered as a choice differed in characteristics other than their eRDP content, including the DUP content, MP yield and food ingredients. Therefore, factors other than eRDP may thus have influenced the diets selected. This hypothesis was therefore tested in Experiments 1, 2, 3 and 4 (Chapters 2, 3 and 4). Once the pH of the rumen environment was thought to be raised through the consumption of $NaHCO_3$, the diets selected by the sheep were in agreement with the results of Tolkamp *et al.* (1998b).

The MP system (AFRC, 1993) used to formulate the foods described in Chapters 2, 3 and 4 does have potential limitations, especially regarding the actual eRDP supply to the sheep. The first limitation of the MP system regarding eRDP is that the calculated eRDP content of a food is generally based on tabulated measurements of N disappearance from nylon bags incubated within the rumen. It cannot be assumed that N disappearance from the nylon bag is synonymous with protein degradation. For example, Waters *et al.* (1992) observed a 20% disappearance of acid detergent insoluble nitrogen (ADIN) from the nylon bag even though ADIN is known to be indigestible. This could therefore result in the diets containing less eRDP than predicted. However, if the diets were deficient in eRDP, then the addition of urea to such foods would rectify the deficiency resulting in increases in rate of intake (Egan and Doyle, 1985; Manyuchi et al., 1994) and live weight gain (Orskov et al., 1972; Kyriazakis and Oldham, 1993). This was the case when urea was added to the food formulated to be deficient (Chapter 3). However, there was no effect of urea supplementation on food intake or live weight gain when added to the foods calculated to contain sufficient eRDP prior to the addition of urea (Experiment 4, Chapter 3). This suggests that these foods did contain adequate amounts of eRDP prior to urea supplementation and, therefore, met the requirements of the experiment.

The second limitation of the MP system regarding eRDP derives from the assumption that the QDP fraction, which includes urea, and the SDP fraction of eRDP are captured

by the rumen microbes with a 'constant' efficiency of 0.8 and 1.0 respectively (ARC, 1980). The amount of protein slowly degraded is determined by the time the food spends in the rumen, which is a function of the level of feeding and outflow rate. Ørskov and McDonald (1979) showed that as outflow rate increased, degradability decreased. In addition, a greater proportion of QDP may be carried from the rumen as outflow rate increases, escaping microbial capture. Therefore as outflow rate increases, due to an increase in food intake, less of the consumed eRDP would be available for capture by the rumen microbes and the efficiency of capture for both QDP and SDP would fall.

The average food intakes of sheep in Experiments 2, 3 and 4 were higher than predicted using dry matter intake calculations based on concentrates (ARC, 1980). Therefore, outflow rate may have been greater than the values used (outflow rate of $0.05h^{-1}$, AFRC, 1993) to calculate the eRDP content of the food. In addition, as a result of a lower degradability, the availability of fME may also have been reduced. However, the efficiency of bacterial growth per MJ fME available increases as outflow rate increases which in turn may offset the decrease in fME availability. Thus, the foods calculated to be eRDP adequate might have, in fact, been deficient.

There are three sets of results that can help disregard the possibility that eRDP was deficient as a result of an increase in outflow rate from the rumen. The first, as described above, was that the addition of urea to such foods would rectify the deficiency resulting in increases in rate of intake and live weight gain. This did not occur. Secondly, sheep showed a preference for the urea-supplemented food when offered a choice, which is consistent with the idea that eRDP was deficient as a result of an increase in outflow rate from the rumen. However, if eRDP was deficient it would be expected that sheep offered, for example, H vs. H+12.5g urea/kg, would select a greater proportion of the urea-supplemented food than sheep offered H vs. H+25g urea/kg in an attempt to rectify the deficiency. This was not the case (Chapters 3 and 4). In fact, sheep offered H vs. H+25g urea/kg selected more of the urea-supplemented food than sheep offered H vs. H+12.5g urea/kg. Finally, supplementing both foods, offered as a choice, with $NaHCO_3$

could increase outflow rate further (Hart and Polan, 1984; Garg and Nangia, 1991; Puri and Kapoor, 1996). Using the above hypothesis that eRDP availability is affected by outflow rate, a further increase in outflow rate would result the food becoming more eRDP deficient. It would be expected that sheep would increase the proportion of the urea-supplemented food in their diet when offered a choice, when both foods offered were supplemented with $NaHCO_3$. However, when $NaHCO_3$ was added to both foods, offered as a choice, sheep decreased their preference for the urea-supplemented foods. It is therefore unlikely that the eRDP content of the food was made deficient through an increase in outflow rate.

The above highlight the importance of using single fed animals as controls in diet selection experiments as it is important that such effects are quantified for the single foods to help interpret the diet selection made by animals offered food choices (Emmans, 1991).

It is not possible to alter the eRDP content of an eRDP deficient food without altering the MP yield, or the ingredients of the food. The experiments reported in this thesis, and those of Tolkamp *et al* (1998b), demonstrate that when the rumen pH is within the desirable physiological range, ruminants avoid an excess consumption of RDP. Although it can be suggested that ruminants select a diet to avoid deficient amounts of eRDP, whether ruminants actually select their diet on the basis of MP or eRDP remains unclear.

Tolkamp *et al.* (1998b) strengthened their argument that ruminants selected their diet on the basis of eRDP and not MP yield by offering cows a choice between two foods that differed in their MP yields but not their eRDP content. The diet selection of these cows did not differ from random. Therefore there was no evidence that the MP yield of foods affected diet selection by ruminants whereas the eRDP content of the foods offered as a choice strongly affected diet selection. Therefore, further research is required to

strengthen the argument that ruminants select a diet to avoid both deficient and abundant concentrations of eRDP.

One possible approach could involve the formulation of a number of foods that differ in both eRDP and MP content. These foods should then be offered two at a time as a choice, in a Latin square arrangement similar to that described in Chapter 2. This would determine if ruminants maintain a diet with an estimated 'optimal' eRDP: fME ratio when the eRDP and MP content of the foods offered change. As in the experiments in this thesis, pelleted foods should be offered to prevent selection within a food that might have occurred in Tolkamp *et al.*'s (1998b) experiment. The results from such experiments, along with information on how the consumption of RDP is monitored and controlled by the ruminant will improve our understanding of what dimensions of the foods ruminant responds to. Such an understanding will subsequently allow present theories of diet selection to be strengthened and new theories to be developed.

6.6 Conclusions

The main objective of this thesis was to address the basis of the diet selection of sheep, with the aim of shedding some light on the rules that ruminants might use when selecting a diet. In view of the importance of microbial protein in the supply of protein to the ruminant host, it was hypothesised that growing sheep might select a diet to obtain an estimated 'optimal' eRDP: fME ratio of 10:1 from two foods that differ mainly in their eRDP content. The 'optimal' eRDP: fME ratio would be expected to maximise microbial protein synthesis, and minimise the energy costs associated with the detoxification of excess ammonia, absorbed from the digestive tract. However, the results from the experiments reported in Chapter 3 did not support this hypothesis. It was suggested that the diets selected by the sheep were, at least in part, a means of reducing the potential disturbance of the rumen environment and had nothing to do with protein either as eRDP or MP. It was possible that urea, used in the experiments reported in

Chapter 3 to alter the eRDP content of the foods, was acting as a buffer. When an alternative buffer was consumed the need for urea, as a buffer, was reduced. The decrease in preference for the urea-supplemented food, and hence an avoidance of an excess consumption of RDP, in the presence of $NaHCO_3$, was then in agreement with the hypothesis that ruminants might select their diet on the basis of eRDP. Further research is required to strengthen the argument that ruminants select a diet to avoid both deficient and abundant concentrations of eRDP.

The results from the experiments reported in this thesis support the hypothesis that one of the objectives of the diet selection of ruminants is to maintain the rumen conditions within a certain physiological range. Therefore, in the absence of long forage, ruminants would be expected to select foods with the greater buffering potential firstly to prevent further disruption to the rumen environment and secondly to return the conditions in the rumen to within the accepted range as soon as possible. This need appeared to override other diet selection objectives such as avoiding an excess intake of RDP (Chapters 3 and 4) or meeting their daily ME requirement (Chapter 5). Maintaining the rumen environment within a certain physiological range appears to underline the basis of ruminant diet selection and makes evolutionary sense, as this would help sustain food intake and yields of energy and protein.

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